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CHEMOPROPHYLAXIS AND ANTIDOTAL EFFICACY OF ALPHA-KETOGLUTARIC ACID IN HYDROGEN CYANIDE POISONING

FINAL REPORT

ARTHUR S. HUME

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A model to study the toxic effect for hydrogen cyanide (HCN) by inhalation and to evaluate the efficiencies and relative potencies of possible antidotes has been established. Several chambers and accompanying mechanics of administration of HCN were designed and evaluated. This model is especially designed to be used to study the effects of HCN at different concentrations and to evaluate the effectiveness of the antidotes with pre- and post- exposure of mice and rats to HCN. In addition, pulmonary and cardiovascular changes in the rat can be monitored. This allows more discrete determination of the efficacy of antidotes and monitoring of toxic effects other than lethality.

During exposure, HCN concentrations in the chamber were continuously monitored by detector tubes, electrochemical sensor, and chemical colorimetric methods. Mice were placed in a cage which was quickly inserted and removed from the chamber. Rats were placed in a rat hold (tube) for insertion of nose-only exposure. An LCT_{50} for mice of 1140 mg/m^3 and an LCT_{80} for mice of 1322 mg/m^3 $\pm$ 5% were determined using this system.

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I. INTRODUCTION

A. Nature of the Problem

Hydrogen cyanide (HCN) is recognized as a rapidly acting, highly toxic chemical. Unconsciousness, respiratory arrest or death can occur so rapidly that post-exposure antidotal intervention is difficult. Therefore, a chemoprophylactic agent which could be administered orally would be highly desirable.

Very little research has been done on antidotes using HCN as the experimental form of cyanide. In most prior research, the effectiveness of the potential antidotal agent has been determined by the reduction of lethality in test animals receiving various cyanide salts. A real need existed to develop a model system in which antidotes were evaluated for their effectiveness in antagonizing the toxic effects of HCN.

Models for the study of toxic effects from the inhalation of HCN have been designed and used. The apparatus of Matijak-Schaper and Alarie (1982) included head only (trachea cannulated and non-cannulated) exposure to HCN, carbon monoxide and low oxygen. Respiration functions were monitored with plethysmographs. An LC_{50} of 166 ppm was reported for HCN in mice. However, this system, as reported, would be partially unsuitable for antidotal research since the animals could not be removed simultaneously from the HCN and the concentrations of HCN in the chamber increased over the exposure time. It would be difficult to assess the differences in treated and non-treated animals.

Two excellent references (Levin et al., 1982; Levin et al., 1983) discuss the technical as well as the toxicological aspects of inhalation of HCN and provide standard conditions for procedures of exposure studies.

Although Ballantyne (1984) did not elaborate on the inhalation method used, he did report that the concentration of cyanide in the blood, at death, of rats which were exposed by inhalation was much less, by comparison, than the concentration of cyanide in the blood of animals that were injected intramuscularly (IM), e. g., 170 mcg CN-/100 ml by inhalation and 746 mcg CN-/100 ml by intramuscular injection.

In addition, there is not a satisfactory chemoprophylactic agent presently available for use against cyanide poisoning. The antidotal regimen of a nitrite and sodium thiosulfate (ST) has serious limitations: i.e., slow response, decreased oxygen transport due to methemoglobin formation, and lack of suitability of route of administration. Also, thiosulfate is rapidly excreted by the kidneys. The nitrites are given only by inhalation (amyl nitrite) or intravenous injection (sodium nitrite [SN]). Other antidotes have been proposed in Europe. Hydroxocobalamin (Vitamin B_{12a}) (HCB) has been used in France and is offered in the U.S. under an Investigational New Drug by the Rocky Mountain Poison Control Center. However, HCB therapy requires tremendously large dosages resulting in some toxicity, is expensive, and its use is limited by its low solubility. Cobalt ethylenediaminetetraacetic acid or cobalt edetate (Kelocyanor®), is available in Great Britain. This chemical is effective but exhibits extensive, unpredictable and serious toxicities. In fact, administration is advised only after cyanide poisoning has been established.

The methemoglobin former, dimethylaminophenol (DMAP) is a rapid and effective antidote for cyanide poisoning; however, it also has serious, toxic side effects and is unpredictable in the production of methemoglobin. P-aminopropiophenone (PAPP) has been shown to exhibit significant anticyanide activity but has a short half-life and is capable of producing deleterious amounts of methemoglobin. Hydroxylamine (HA) is very rapid in the onset but is erratic in the induction of methemoglobin formation.

Thus, the search for a more adaptable, efficacious antidote for cyanide poisoning continues. Our laboratory has done extensive research with alpha-ketoglutaric acid (AKG). This chemical is equally as effective as nitrite/thiosulfate (Moore et al., 1986). In combination with thiosulfate, AKG is three times as effective by weight in reducing lethality, as nitrite/thiosulfate administered prophylactically against ionic CN.

B. Previous Work by Principal Investigator

1. Chemoprophylaxis of Cyanide Poisoning by Thiol- and Sulfur-Containing Compounds

Thiol- and sulfur-containing compounds were investigated for their abilities to prophylactically prevent death from CN poisoning. Of the number of compounds evaluated, methionine, cystine, cysteine and N-acetylcysteine were found to be significantly effective as prophylactic agents against the lethality of cyanide in LD_{90} doses. On the other hand, the sulfhydryl-containing compounds 2,3 dimercaptopropanol, disulfiram and methane sulfonic acid were ineffective in chemoprophylaxis of potassium cyanide (KCN) poisoning (Benet et al., 1983; Benet et al., 1984).

2. Chemoprophylaxis of Cyanide Poisoning by Carbonyl Compounds

The carbonyl compounds pyruvic acid, ascorbic acid, dehydroascorbic acid, pyridoxal hydrochloride, AKG and β-ketoglutaric acid (BKG) were evaluated for their abilities to prophylactically prevent death from KCN. The studies showed that pyruvic acid, dehydroascorbic acid and AKG had significantly effective prophylactic abilities against CN poisoning, as did orally administered ascorbic acid. Pyridoxal hydrochloride and BKG did not protect mice against lethal doses of KCN. The LD_{50} value of KCN was increased to 35.0 mg/kg when AKG was administered prophylactically at a dose of 2 g/kg; this represents a six-fold increase in KCN dosage which resulted in an LD_{50} (Aldous et al., 1984).

The hypothesis is offered that A-keto acids bind cyanide, forming cyanohydrins, which inhibit the availability of CN at a cellular level to the extent that lethality is prevented. This hypothesis is supported by in vitro and in vivo studies reported by our laboratory (Norris and Hume, 1986).

3. Additional Studies on Chemoprophylaxis of CN Poisoning by Alpha-ketoglutaric Acid

In studies done by our laboratory, it was shown that while AKG alone in a dose of 2 g/kg, IP, is as effective as sodium nitrite (SN) (100 mg/kg) and ST (1 g/kg) in protecting mice against the lethality of CN, a combination of ST and AKG protected in a measure three times greater by weight in reducing lethality than a combination of SN and ST (Moore et al., 1985; Moore et al., 1986). In studies done in our laboratory to evaluate the effectiveness of orally administered AKG, it was observed that all doses of AKG were significantly effective in decreasing the toxic symptoms of CN poisoning as well as in reducing the lethality from CN (Dulaney et al., 1987).

4. Studies on Prevention of CN Induced Inhibition of Cytochrome Oxidase (Cytox) by A-ketoglutaric acid

In both in vivo and in vitro studies, it was shown that inhibition of brain cytochrome oxidase activity by cyanide can be prevented by AKG (Norris et al., 1986).

C. Previous Work by Other Investigators

To be of value, treatment for cyanide poisoning must occur immediately after intoxication. A lethal dose of approximately 156 mg of cyanide can be bound when 10% of an adult's hemoglobin is in the metheme state (Baumeister et al., 1975). The optimum therapeutic level of methemoglobin is estimated to be 25%; however, if inexorable manifestations of cyanide toxicity persist, levels of 40% can be induced (Peters et al., 1982). Methemoglobin levels of 30% or less seem to produce no overt symptoms, while levels of 60-70% can be considered to be lethal (Bodansky, 1951). However, Guertler, et al., (1991) reported that methemoglobin levels greater than 10% resulted in significant decrease in performance in sheep.

Kiese and Weger (1965) have recommended the use of aminophenols in cyanide intoxication. DMAP has been shown to produce methemoglobin at a much more rapid rate than sodium nitrite (Way et al., 1984). DMAP has also been reported to have a much less action upon circulation and respiration as compared to nitrite (Kiese and Weger, 1965). However, Kruszyna et al. (1982) maintain that cyanide is released more rapidly from

cyanomethemoglobin in animals pretreated with DMAP than in animals pretreated with sodium nitrite. Thus, they reported animals surviving the initial cyanide insult dying 30-60 minutes after cyanide injection.

Cobalt edetate (Kelocyanor®) has been suggested as the treatment of choice in cyanide poisoning (Lancet, 1977) and is the preferred cyanide antagonist in Great Britain (Way et al., 1984). The efficacy of cobalt compounds in the protection against cyanide intoxication has been known for many years and was reported by Way et al. (1984) and by Lang (1895). This agent acts by expeditiously chelating tissue-bound and free plasma cyanide (Peters et al., 1982). Its main advantage as a cyanide antidote is that it does not affect the oxygen-carrying capacity of the blood as do the methemoglobin-forming agents. Sodium thiosulfate has been reported to cause a more-than-additive increase in the antidotal effectiveness of the cobalt chelators (Frankenberg and Sorbo, 1975). The main disadvantage of the cobalt chelators is that they may cause ventricular arrhythmias (Way et al., 1984). Anaphylactoid reactions have also been disclosed in cobalt treatment, such as neurotic edema of the face and neck (McKieran, 1980). Cobalt edetate treatment can cause gastrointestinal hemorrhage, hypotension with transient tachycardia, skin rash, and vomiting (McKieran, 1980).

HCB has been proposed for use in cyanide poisoning (Mushett et al., 1952; Delga et al., 1961). Its effectiveness appears to be due to its ability to bind cyanide, resulting in the formation of cyanocobalamin (Peters et al., 1982). Ivankovich et al. (1980) report that the dose of HCB necessary to provide protection against cyanide intoxication in the dog was of sufficient quantity to produce a reddish skin discoloration. They also reported that the animals were not maintained in a viable cardiovascular state by HCB therapy.

Cyanide is a potent nucleophile and may react with carbonyl groups to form cyanohydrins (Morrison and Boyd, 1973). Green and Williamson (1937) ascertained that pyruvic acid, an a-keto acid, reacts with cyanide in vitro to form pyruvic acid cyanohydrin. Cittadini et al. (1972) have shown that sodium pyruvate is effective against cyanide intoxication. Way et al. (1984) maintain that, since this compound is actively transported, it is more likely to be distributed to areas of the body wherein cyanide is localized. However, sodium pyruvate affords only minimal protection against the lethal effects of cyanide, and alone is much less effective than either sodium thiosulfate or sodium nitrite (Schwartz et al., 1979).

K. P. Ivanov (1959) of the I. P. Pavlov Institute of Physiology, USSR, reported on the effectiveness of elevated oxygen pressure on animals poisoned with potassium cyanide. This work was complemented by studies on the protective effect of oxygen against cyanide intoxication performed by Way et al. (1966) in the U.S. Way et al. (1966) found that oxygen exerted only a minimal, if any, protective effect against cyanide intoxication. However, this group discovered that oxygen prophylactically increased the antidotal efficacy of ST (Way et al., 1966a) and, more important, therapeutically enhanced the antidotal efficacy of ST (Sheehy and Way, 1968). Although oxygen enhanced the antidotal efficacy of ST to a "minor degree," it provided potentiation of the efficacy of the antidotal combination of ST and SN (Way et al., 1984).

II. PURPOSE OF THE WORK

The primary purpose or objective of the study was to establish and validate an inhalation model to be used to evaluate antidotes in animals exposed to gaseous HCN.

It also became a purpose of this project to develop precise, accurate concentrations of HCN in the chamber. This concentration must be reproducible if antidotal work (protection ratios) is to be very significant. Such a model was of particular importance since so little research had been reported in antidoting the toxic effects of HCN by inhalation.

Another purpose of this work was the establishment of a model for exposure in which heart rate (HR), blood pressure (BP), and blood CN levels could be monitored while the animals were being exposed to HCN.

Once the model for exposure of animals was established, potential antidotes were evaluated. These potential antidotes were administered IP or IM in order to evaluate their effectiveness against the toxic effects of

gaseous cyanide. The effect of the route of administration of cyanide upon the efficacy of anticyanide agents was evaluated.

It was important that a hydrogen cyanide model be developed for this form of cyanide because, although most of the literature that has been published has involved cyanide salts, the potential exists for the use of HCN by a foreign power or by terrorists against the U.S.

III. GENERAL METHODS

In establishing a model using a gas (HCN) which is highly toxic and rapidly active, development of safe working conditions was the first task. Second, the effluent gases had to be cleaned of waste HCN prior to discharge into the atmosphere. Third, the inhalation chamber must be designed and constructed to accomplish the objective of the project. EPA guidelines were used in establishment of this model.

Mice and rats were exposed to measured concentrations of HCN for definite time intervals with accurate dosing of the animals and good reproduction of experimental conditions. Cannulations of femoral vein and artery in rats provided access to blood samples and allowed monitoring of cardiac parameters. Blood samples were analyzed for cyanide content. The ability to monitor HR and BP allowed us to monitor the toxic effects of cyanide more closely so that the effectiveness of antidotes could be evaluated in animals being exposed to cyanide.

A. Establishment of Apparatus Necessary to Expose Animals to Hydrogen Cyanide

1. Preliminary Work on Hydrogen Cyanide Exposure Chamber

Considerable delays in renovating the existing exhaust hood were experienced. It is estimated that a loss of some 5 months resulted from these delays. The delays were attributed to inexperience of the contractor in the design and construction of the cyanide scrubbing system.

As can be seen in Figure 1, the removal of waste gaseous HCN from the exhaust hood was accomplished by an extensive exhaust system. The renovated hood system included a sodium hydroxide scrubbing system through which the exhaust gas was passed to remove HCN before being released into the atmosphere. A high volume exhaust fan, producing a flow of 1175 cu ft/min (33.3 m^3/min) was installed to provide a system in which little or no HCN would be allowed to escape into the laboratory.

To further ensure safety, an HCN detection system was provided with sensors located in the laboratory and on the roof adjacent to the scrubber, and in the exhaust gas in order to monitor possible HCN leakage. The system worked well, with no HCN alarms occurring from the detection of HCN gas during this course of study.

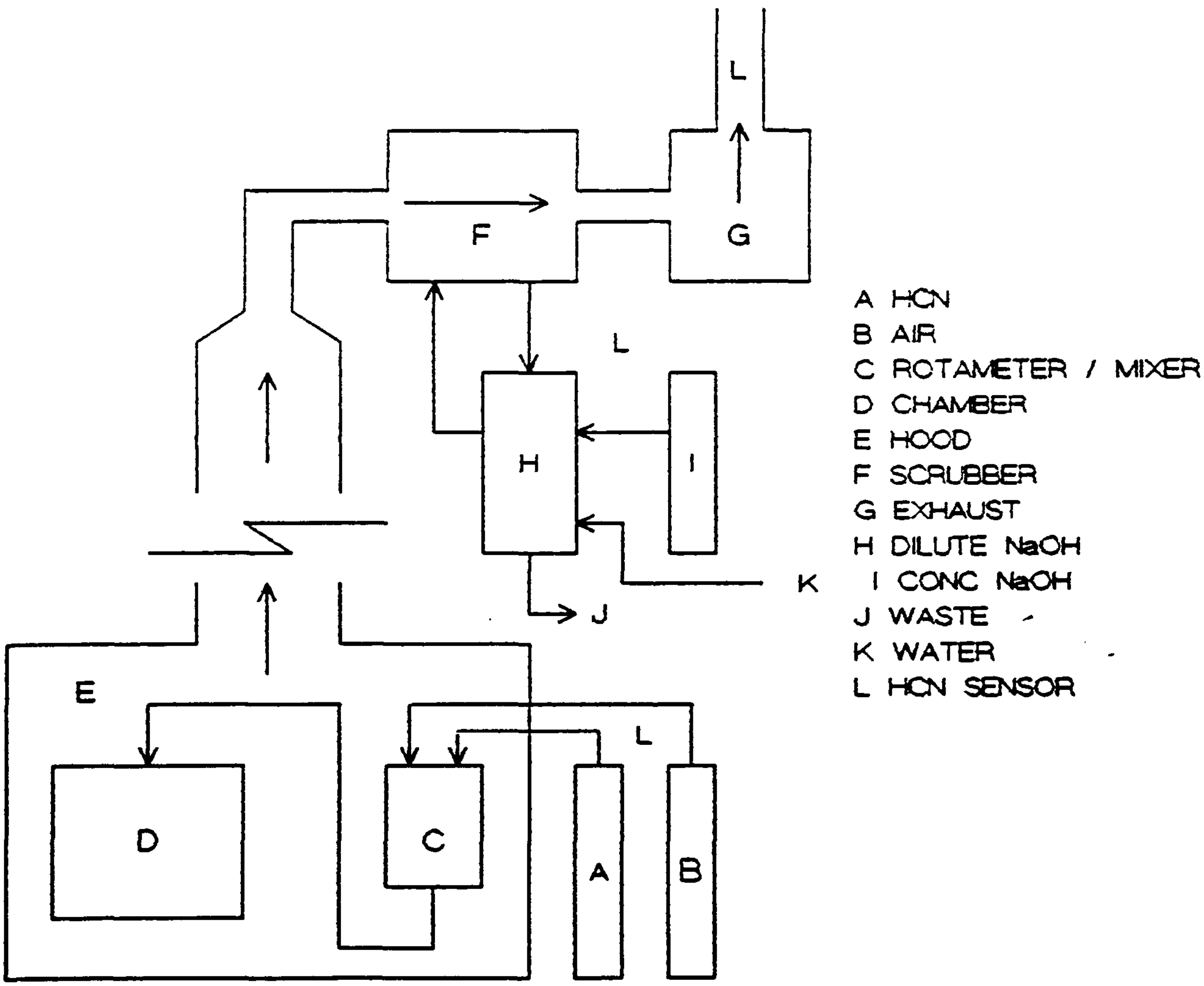
Experiments in which HCN was produced in a static test chamber gave nonreproducible results. NaCN was allowed to react with an excess of H_2SO_4 in a sealed chamber equipped with a sample port and fan. Varying the amount of NaCN (0.1 g, 0.2 g, 0.45 g, and 0.908 g NaCN) with excess H_2SO_4 produced HCN, but in unpredictable amounts. Relocation of the HCN generator outside the chamber did not give better results.

Concentrations of HCN in the static chamber were determined by use of a flow meter and U-tube in a dry ice-acetone mixture, trapping the HCN. At a rate of 200 ml/min, 0.05 g of HCN was collected in the U-tube after 10 minutes. Calculations showed the concentration of the gaseous HCN in the chamber to be 14,793 mg/m^3.

It appeared feasible that samples of chamber ambient air containing HCN could be collected with a gas-tight syringe, added to an alkaline solution, and the HCN content quantitated by means of a CN ion electrode. This procedure proved to be too slow, and the selective ion (CN^-) electrode failed to indicate the correct value.

FIGURE 1

HCN INHALATION SYSTEM



By limiting the time of flow of the HCN, various concentrations of HCN could be achieved in the static chamber. The results are shown below.

Run No.	No. Mice	Exposure time (sec) (flow at 200 ml/min)	HCN Conc. (mg/m^3)	Lethality %
1	5	20	494	0
2	5	25	617	100
3	4	22	543	75
4	5	20	494	0

Although the initial concentration of HCN could be calculated and is shown above, the actual concentration during the exposure could not be reported on a reliable basis due to the influx of air when the mice were introduced.

2. Design of the Dynamic Inhalation Chamber for Exposure of Mice to Hydrogen Cyanide

Figure 2A depicts the first dynamic inhalation chamber designed to expose mice to hydrogen cyanide. The chamber employed was rectangular in shape, having the dimensions of 16.8 inches x 12 inches x 4.75 inches. (15.7L) The chamber was divided into two compartments which were separated from each other by a sliding door. The outer chamber encompassed 47 square inches of floor space, while the larger inner chamber encompassed 144 square inches of floor space. The outer chamber served as a holding station for the animals immediately prior to their introduction into the inner chamber. Animals were placed into the holding chamber when the inner compartment's atmosphere was determined to contain the desired concentration of each gas, and the door was opened. The inner chamber was sampled subsequent to the introduction of the animals (and closing of the sliding door) in order to measure any change in gas concentration which might be attributed to the introduction process itself. Intermittent gas sampling was performed throughout the exposure period to assure that the particular gas mixture remained unchanged throughout challenge. The use of this chamber was discontinued because of the difficulty in controlling the HCN concentration which occurred when the sliding door was opened. The apparatus in Figure 2B was constructed. In this device, mice were dropped via a trap door, into an exposure chamber similar in size to the previous chamber 2A. However, this apparatus also allowed too much atmospheric air to enter the chamber with subsequent loss of control of HCN concentration. This was the first chamber in which continuous monitoring of HCN was accomplished.

The final design (Figure 2C and Figure 3) was a much larger chamber (125 L). This large volume diminished the effect of atmospheric air entering the inhalation chamber. The mice were introduced into the chamber via a screen cage which allowed exact exposure times and limited their mobility (Figure 4A).

Since hydrogen sulfide has been reported as a component of mouse urine, efforts were made to minimize this contaminant, particularly in our determination of chamber concentration of HCN. The electrode responsible for monitoring HCN concentration was poisoned by small amounts of sulfides and responded by indicating lower concentrations of HCN than were actually present. Installation of a sulfide/sulfate filter on the electrode alleviated this problem. Fans installed in the rear and side of the inner chamber aided in the maintenance of a homogeneous gas mixture (no dead space in the chamber).

3. Design of the Inhalation Chamber for Exposure of Rats to Hydrogen Cyanide

Figure 5 depicts the dynamic inhalation chamber designed for the exposure of a single rat to HCN. The chamber is rectangular in shape, having the dimensions of 25.5 cm x 30.5 cm x 25.5 cm (19.8L). The chamber is fitted with inlet and outlet ports for the administration of HCN, a HCN monitor electrode (equipped with a sulfide/ sulfate filter), sample port, circulating fan, rat immobilizer sleeve, and door.

FIGURE 2

DEVELOPMENT OF HCN CHAMBER FOR MICE

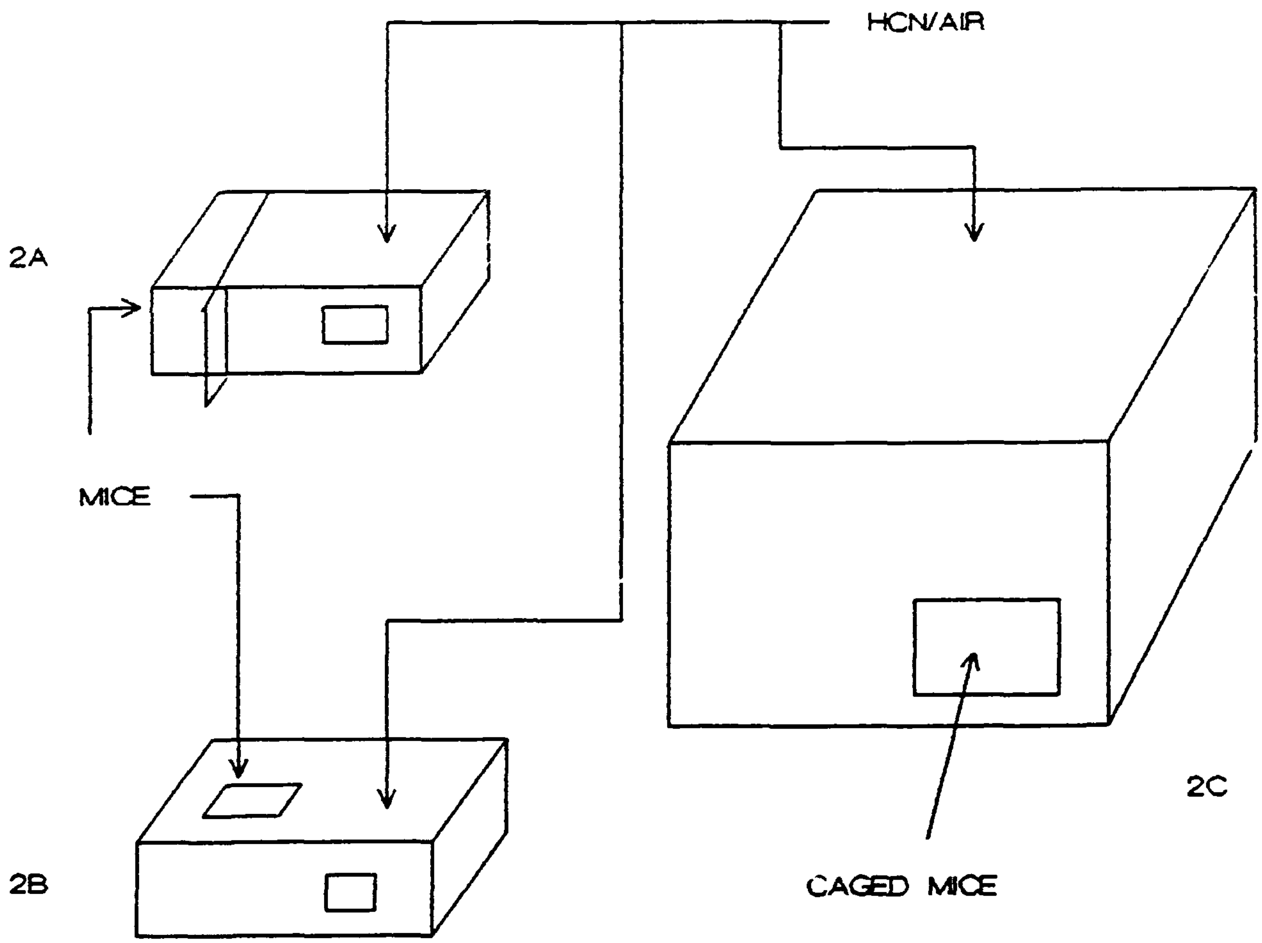
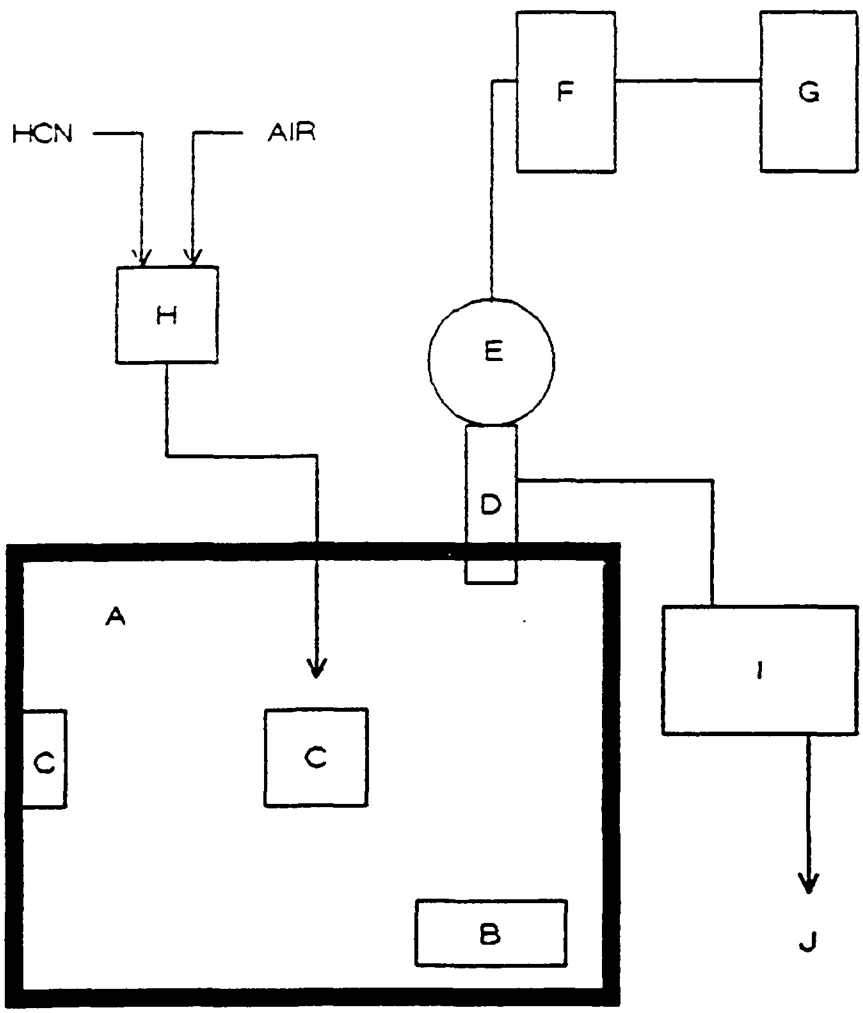


FIGURE 3

HCN INHALATION CHAMBER
FOR MICE

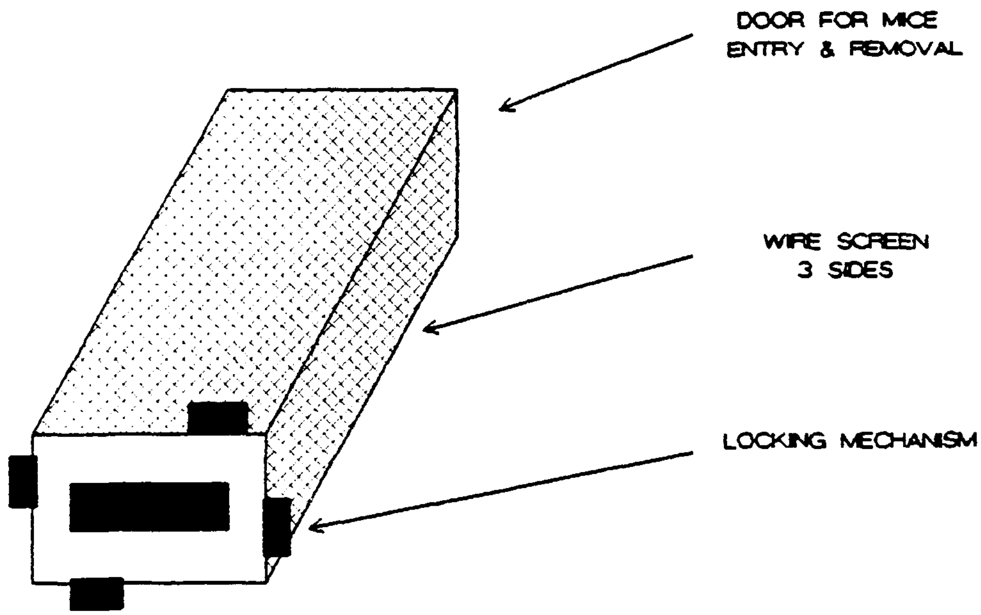


A INHALATION CHAMBER
B CAGE ENTRY
C FAN
D SENSOR / FILTER
E ELECTRONICS
F VOLTMETER
G POWER SUPPLY
H FLOWMETER / MIXER
I PUMP
J DETECTOR TUBE

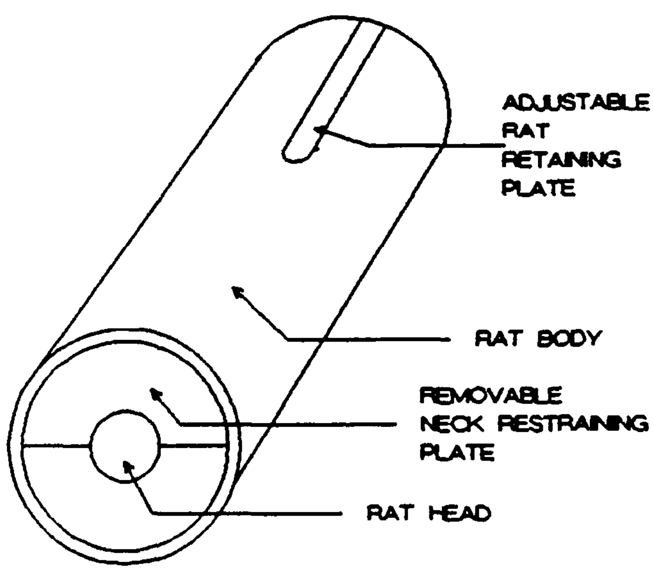
FIGURE 4

RESTRAINING DEVICES FOR MICE & RATS

4A



4B



The rat immobilizer (Figure 4 B) is made of a 7.5-cm-in-diameter aluminum tube. It is fitted with dual O-rings which are matched to produce a tight fit into the rat immobilizer sleeve. The immobilizer has grooves at the top and bottom for catheters and monitor lines. The immobilizer is constructed so that a circular opening of 2.0 cm in diameter is located 1.0 cm forward of the O-ring. The small opening allows only a portion of the rat's nose to be exposed to the HCN, thereby reducing the area of HCN absorption through the skin and animal hair.

The HCN is introduced via rotameters and mixer into the chamber through the inlet port. A sample port is provided, allowing the contents of the chamber to be sampled at any time for HCN or oxygen analyses. When the desired concentration of HCN is reached as indicated by the HCN monitor and confirmed by HCN detector tubes, the rat, already contained in the immobilizer, is placed into the immobilizer sleeve. Thus, the animal can be exposed to a known concentration of HCN for a determined length of time in order to determine LC values. In addition, when the rats are catheterized, cardiovascular variables can be monitored and blood samples can be collected at any time for analysis of cyanide or other measurements.

4. Analysis of Hydrogen Cyanide from the Inhalation Chamber

Initially, our plans were to standardize the HCN concentration in the inhalation chamber by purchasing commercially available mixtures of HCN and helium. Two shipments of HCN (200 ppm) by assay were received from Matheson. One tank, after much deliberation and system checks, was found to contain 440 ppm and was returned to the manufacturer. The replacement tank was also in error as it contained only 100 ppm of HCN. The inability of Matheson to provide a standard gas convinced us to devise additional methods for analysis for HCN in the chamber.

Sensidyne, Inc. (Clearwater, FL) furnishes Gastec® HCN detector tubes in the range of 50-800 ppm. When used to measure HCN concentrations of 17-2400 ppm by controlling the volume of the sample, they will exhibit a maximum inaccuracy ± 25%. The same tubes, however, in our laboratory, produced an accuracy of ± 2% in the HCN concentration range of 360-425 ppm when the results were read at 2 minutes exposure time. Colorimetric analyses of HCN as described elsewhere, confirmed this degree of accuracy.

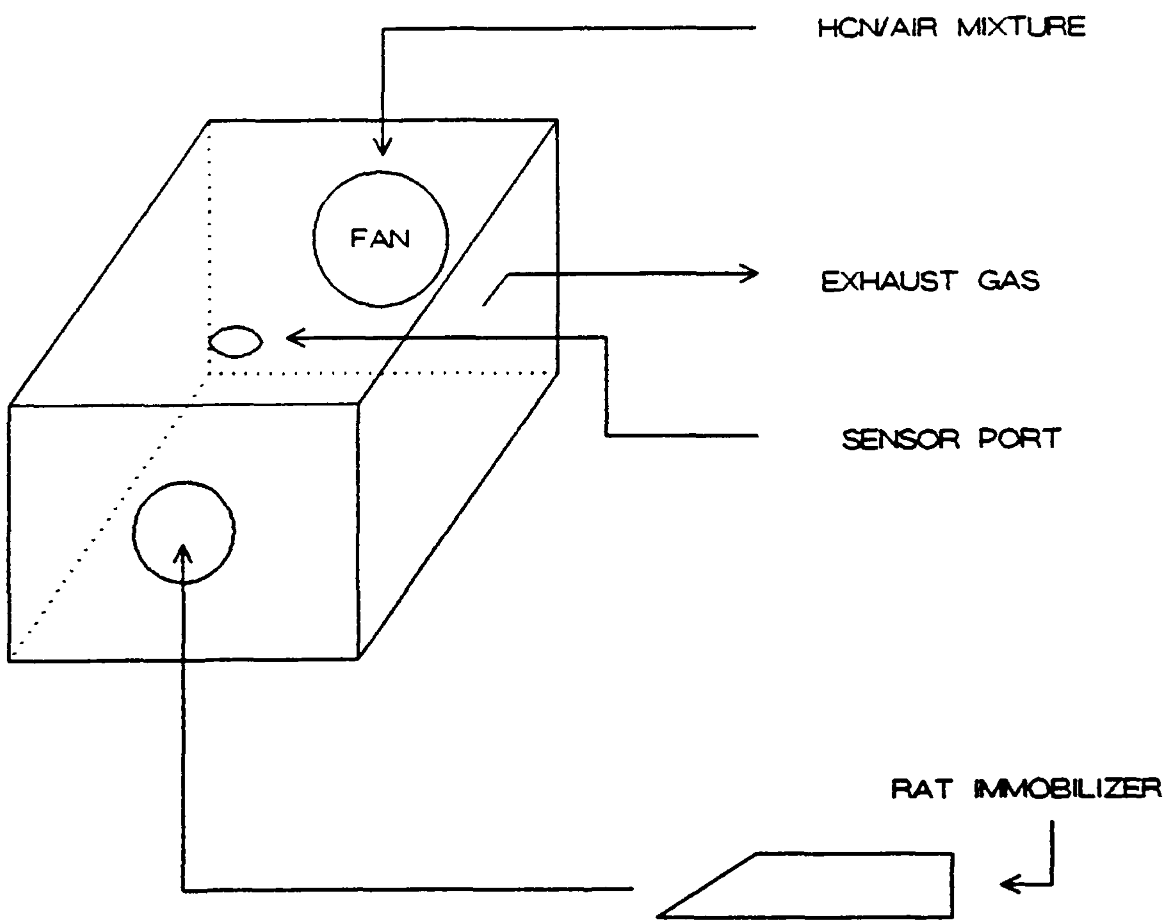
To establish and maintain a constant concentration of HCN in the chamber, we used the following (1) assayed HCN gas mixture (10000 ppm), (2) rotameters for measurement of flows of HCN/He and breathing air in the proper proportions, (3) CN electrode sensor in chamber, (4) CN detector tubes on effluent gases, (5) colorimetric assay of CN concentration in support of other measurements.

By use of rotameters and a non-corrected HCN electrode, the desired approximate HCN concentration of the chamber could be achieved. A sample of the exhaust gas was then drawn through the HCN detector tube, and at 2 minutes, the tube results were read. The HCN electrode was then calibrated to the correct mA reading as shown by the gas detector tube. When plotting the output current (mA) of the HCN sensor against concentration of HCN (ppm) a curve results. The equation of the curve is: mA = 4.6 + 7.7 log [HCN] (HCN concentration in ppm) (Sensidyne Instruction Manual). The HCN electrode readings were monitored throughout the time of animal exposure. A variation of 0.1 mA indicated a change of approximately 10 mg/m^3 HCN concentration. To further ensure correct HCN concentrations, HCN was measured by gas detector tube during all experiments.

To analyze the HCN in the chamber by another method, a 300 ml volumetric gas tube was immediately attached to the exhaust gas outlet for 3 minutes. The tube was removed and the contents flushed into 30 ml of 0.1 m NaOH for 10 minutes. 100 μl of this sample was placed into a screw cap tube containing 1.0 ml of NaH_2PO_4. Chloramine T (0.5 ml) was added and allowed to stand for 1 minute. A solution of barbituric acid and pyridine (1.5 ml) was added and allowed to stand for 10 minutes. The light purple solution was transferred to a cuvette and read on a spectophotometer at 580 nm. The absorption of this solution was compared to absorption of standard solutions to determine the CN^- concentration.

FIGURE 5

RAT INHALATION CHAMBER



B. Analyses of Blood for Cyanide Content

1. Analysis of Blood samples for Cyanide Using Microdiffusion

A modification of the method of Feldstein and Klendshoj (1954) was established in our laboratory for the determination of CN content. Sodium hydroxide solution was placed in the center well of a Conway diffusion cell. The blood sample was then placed in the outer compartment, acidified with sulfuric acid and the cell sealed for 3-4 hours. A sample was taken from the center compartment, buffered with phosphate and allowed to react with Chloramine T for 2-3 minutes. After the addition of a pyridine-barbituric acid solution, the mixture was allowed to stand for 10 minutes and placed in a visible spectrophotometer, and the absorption at 580 nm recorded. Calculation of concentration of CN was made using a response factor derived from a standard curve.

2. Determination of Whole Blood Cyanide Concentrations Using Gas Chromatography

A modification of the method of Darr *et al* (1980) has been established in our laboratory. One-tenth of a milliliter of concentrated phosphoric acid was placed into a one milliliter silanized reaction vial. The vial was subsequently capped, and two-tenths of a milliliter of heparinized blood was injected through the septum. The acid-treated blood samples were vortexed for 30 seconds and then were placed into a $60^{O}C$ water bath for 60 minutes. Five hundred microliters of vial headspace were injected into a Hewlett-Packard series 5880A gas chromatograph equipped with a nitrogen phosphorous detector. Quantity of CN is determined by comparison with results of analysis of standard CN solutions.

3. Analysis of Cyanide Using Ion-Selective Electrode (The Orion Research, Inc.)

The cyanide electrode (9406BN) was evaluated under laboratory conditions to determine whether or not it would perform satisfactorily in determining sodium cyanide concentrations. After a 1:100 dilution of the sodium cyanide solution, and without a large excess of sodium hydroxide solution to stabilize the mixture, excellent results were obtained. However, the transition to determine the HCN concentrations in blood gave erroneous results and this method was discarded.

C. Determination of Efficacy of Antidotes Against Parenterally Administered Cyanide

Groups of animals (10 mice per dose) were administered three different doses of cyanide in order to generate a lethality curve for each pretreatment protocol. The sodium cyanide was calculated on a CN basis for LD_{40} (6.0 mg/kg), LD_{60} (6.8 mg/kg) and LD_{80} (7.1 mg/kg). Volumes of the injected solutions were kept small (0.1 ml/10 g body weight), and the cyanide solution was always injected antipodal to the peritoneal region into which the cyanide antagonists had been injected in order to decrease any likelihood of intraperitioneal binding between the cyanide and cyanide antagonists. LD_{50}'s were determined over a period of 24 hours after cyanide challenge.

All LD_{50} values were determined by Statistical Analysis System (SAS) utilizing the methods of Finney (1971) or Litchfield and Wilcox (1949). A significance of $P<0.05$ is acceptable.

D. Procedures for Catheterization of Rats and Monitoring Cardiovascular Parameters

1. *Procedure for Catheterization of Rats*

Sprague-Dawley rats, 300-400 g (body weight) were anesthetized by the administration of halothane via an inhalation apparatus. Cannulas were placed in the left femoral artery or vein. The cannulas were then run up to the nape of the neck and out through an incision which prevented the animal from biting or chewing the cannula. Butorphanol was administered to the animals post-operatively. The cannulas were flushed with heparin/saline to maintain patency. Injections of cyanide solutions or antidote were made through the venous

catheter. Also, blood samples were collected readily for cyanide analysis. The arterial cannula was connected to the pressure transducer for monitoring HR, MBP and BP.

2. Procedure for Monitoring Cardiovascular Parameters

Rats, Sprague-Dawley, weight 300-400 g, were cannulated surgically. One day after cannulation, the catheters were tested for patency. If patency and animal conditions were acceptable, one animal was placed in the rat immobilizer, which allowed access to the catheters. The arterial catheter was connected to the pressure transducer via a polyethylene (PE) tube. The transducer was connected to the Grass Multichannel Polygraph (Model 7). The polygraph was calibrated to record MBP, BP, and HR simultaneously. A control non-treated response was recorded. A solution of sodium cyanide was injected (2 mg/kg over 22 seconds) through the venous catheter. MBP, BP and HR were monitored for the toxic effects of cyanide. The severity and extent of the toxic effects on MBP, BP and HR were noted. Then the effectiveness of the antidotes in preventing or alleviating these toxic symptoms was noted and recorded. This system allowed the monitoring of CN induced cardiovascular toxic effects in the conscious animals. This procedure also allows the evaluation of an antidote administered after the toxic effects of cyanide are present. This is most important if antidotes are to be developed for treating the cyanide intoxicated patient since the cardiotoxic effects must be reversed or alleviated.

IV. RESULTS

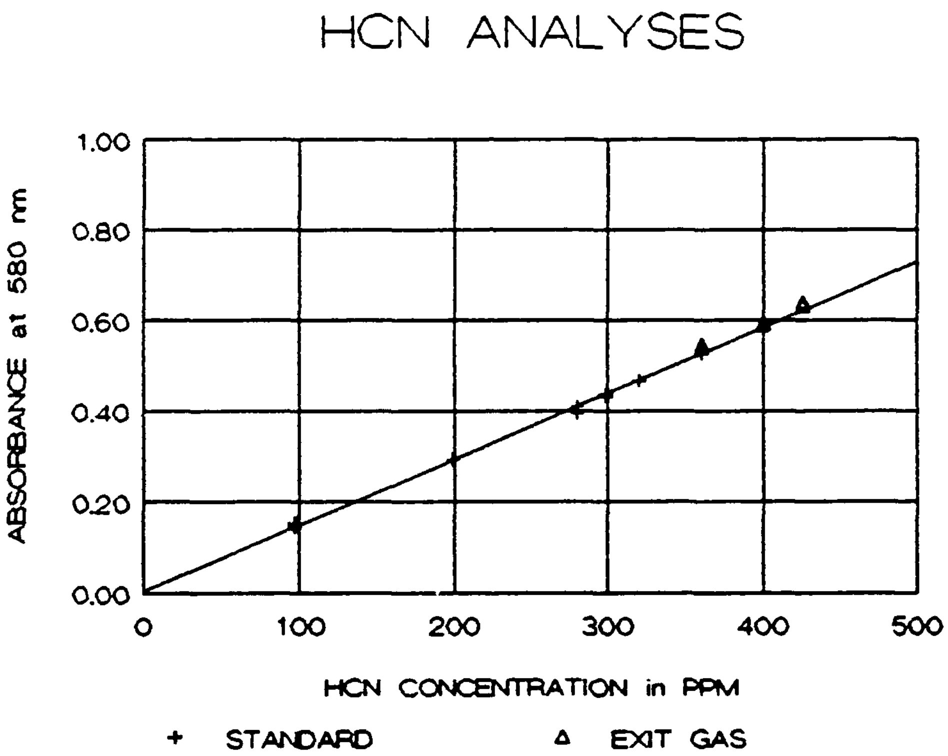
A. Establishment and Validation of Inhalation Chamber

Several inhalation chambers were constructed during the development of the toxic gas system. The final system consisted of a 125 liter cube constructed of 12 mm Plexiglass fitted with a removable door, gas inlet, exit, sample port, two circulating fans, and an orifice for the HCN monitoring electrode. The HCN electrode was fitted with a sulfide/sulfate filter which was connected to a peristaltic pump to provide constant air flow past the monitor (Figure 3). After two attempts to acquire an analytical standard of HCN from Matheson failed because of faulty assays, calibrations of the monitor were accomplished by HCN detector tubes on the exit gases and analyses of HCN by collection and assay method. The % error of the tubes was decreased to less than 2% by reading the results at two minutes. This finding was confirmed by analysis as shown in Figure 6.

B. Determination of LD_{50} Values for Sodium Cyanide with and without Antidotes

To evaluate antidotes and their ability to prevent symptoms and lethality of cyanide, injections of the respective antidotes were made at different doses into the right abdomen, contra to the injection of sodium cyanide solution. Animals were observed for onset of symptoms, severity of symptoms and death. Results were calculated according to the statistical methods found in the section on the LD_{50} determination of cyanide.

FIGURE 6



Samples of the exit gas containing 360, 400, and 425 ppm HCN by gas detection tubes were analyzed for HCN concentration.

Results of a study in which antidotal agents were administered on a molar basis are listed in Table 1. Kelocyanor, hydroxocobalamin, alpha-ketoglutaric acid, sodium nitrite and sodium thiosulfate were administered in 2:1, 4:1, 5:1 and 20:1 molar ratios of antidote: cyanide. All antidotes were effective in protection against lethality at LD_{40}, LD_{70} and LD_{90} doses of NaCN. However, at a 20:1 ratio, the required 200 mg/kg dose of sodium nitrite was lethal in 100% of the animals tested.

On an equimolar basis, AKG, dicobalt edetate (CoEDTA), and HCB provided complete protection at these dosages of NaCN administered parenterally.

In Figure 7, AKG is shown to increase the LD_{50} of potassium cyanide (KCN) from 6.47 to 10.97 mg/kg at a dose of 200 mg/kg IP. AKG was compared to HCB at a cyanide dose of 9.70 mg/kg and the protection was approximately the same. Also, when sodium thiosulfate is added to AKG and HCB, the LD_{50} of cyanide is increased to 26.05 and 25.85 mg/kg, respectively (Figure 8). It is apparent from these results that AKG and HCB are quite comparable in efficacy as antidotes for the toxic effects of cyanide.

Blood cyanide levels in rats were obtained after intravenous administration of 0.5, 1.0 and 2.0 mg/kg of KCN (Figure 9). In Figure 10, the single value for KCN alone represents a blood sample collected at the time of death of the animal. The wide variability in the results as observed in figures 9 and 10 are expected since the doses and rate of injection are different. However, some significance can be assigned to the survivability and constant blood CN^- level in the AKG treated animals.

Additional studies on rats with NaCN and HCN were accomplished. In the experiments, rats were injected IV over 22-25 seconds with two concentrations of NaCN. In the study, 2.4 mg/kg of NaCN was injected to one group and 0.5 mg/kg to the other to produce the following mean cyanide blood level concentrations.

Conc. of NaCN	CN blood level 1.0 min	CN blood level 3.0 min	CN blood level 10.0 min
2.4 µg/kg	6.67 µg/ml	6.98 µg/ml	7.13 µg/ml
0.5 µg/kg	1.94 µg/ml	1.81 µg/ml	1.25 µg/ml

With NaCN, all rats recovered. However, this was not the case with inhaled HCN. Even though cyanide blood levels were much lower than one group of the NaCN injected rats, all rats receiving the inhaled HCN died within 8.5 minutes (Figure 11).

Conc. of HCN	CN blood level 1.0 min	CN blood level 3.0 min	CN blood level 8.5 min
441 mg/m^3	3.03 µg/ml	3.78 µg/ml	4.03 µg/ml

Cardiovascular monitoring procedures have been developed in our laboratory. Rats were catheterized as described elsewhere. The arterial catheter was connected via transducer to a 2 pen recorder. A typical control was observed in Figure 12, in which HR and MBP were monitored. The catherized rats in this experiment were allowed to equilibrate in the holding tube and then were exposed to air or HCN. A concentration of 441 mg/m^3 HCN produced a rapid drop in heart rate with the blood pressure first increasing and then rapidly decreasing until death at approximately 8 minutes. The antidote, SN, was given 30 minutes prior to HCN exposure and produced an overall lowering of blood pressure and heart rate but offered the animal complete protection for over 20 minutes. The rapid decrease in heart rate and the slight increase and then rapid decrease in blood pressure appeared to be typical of rats exposed to HCN (Figure 13).

TABLE 1

EFFECTIVENESS OF ANTAGONISTS TO LETHALITY OF CYANIDE IN MICE

ANTAGONIST	DOSE (ANTAGONIST)		DOSE (NaCN)		MOLAR RATIO	LETHAL %
	MG/KG	mM/KG	MG/KG	mM/KG		
Kelocyanor*	182.0	0.45	4.5 (LD40)**	0.09	5:1	0
Hydroxocobalamin	620.0	0.45	4.5	0.09	5:1	0
Sodium nitrite	30.9	0.45	4.5	0.09	5:1	0
α-Ketoglutaric acid	85.2	0.45	4.5	0.09	5:1	0
Sodium thiosulfate	111.3	0.45	4.5	0.09	5:1	0
Kelocyanor*	182.0	0.45	5.5 (LD70)**	5.5	4:1	0
Hydroxocobalamin	620.0	0.45	5.5	0.11	4:1	0
Sodium nitrite	30.9	0.45	5.5	0.11	4:1	0
α-Ketoglutaric acid	85.2	0.45	5.5	0.11	4:1	0
Sodium thiosulfate	111.3	0.45	5.5	0.11	4:1	0
Kelocyanor*	91.0	0.22	5.5	0.11	2:1	0
Sodium nitrite	15.5	0.22	5.5	0.11	2:1	0
α-Ketoglutaric acid	42.6	0.22	5.5	0.11	2:1	0
Sodium thiosulfate	55.7	0.22	5.5	0.11	2:1	0
α-Ketoglutaric acid	551.0	2.90	7.1 (LD90)**	0.15	20:1	0
Sodium thiosulfate	719.0	2.90	7.1	0.15	20:1	0
Sodium nitrite	200.0	2.90	0.0	0.00	20:0	100

* Kelocyanor - dicobalt edetate

** LD values for NaCN were calculated according to Litchfield and Wilcoxon (1949).

FIGURE 7

EFFECT OF AKG ON LD_{50} OF KCN

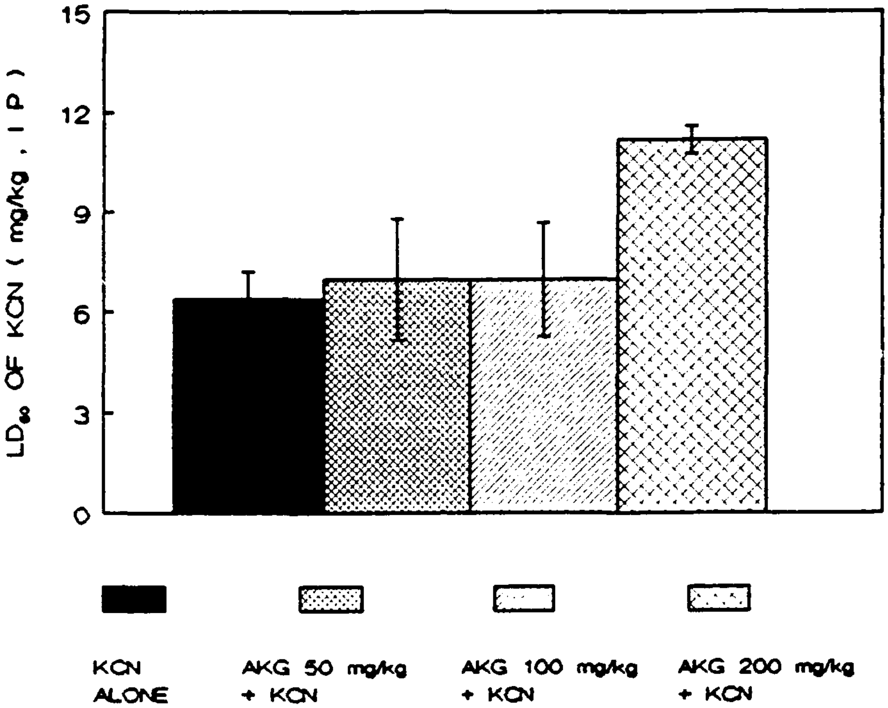


FIGURE 8

EFFECT OF ST, AKG, & HCB ON THE LD_{50} OF KCN

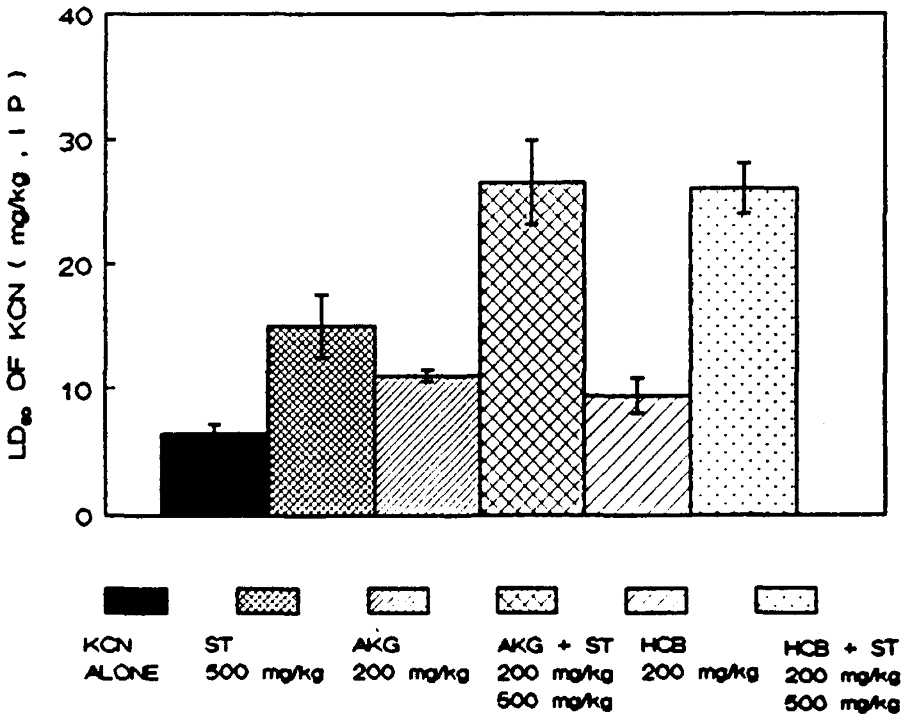
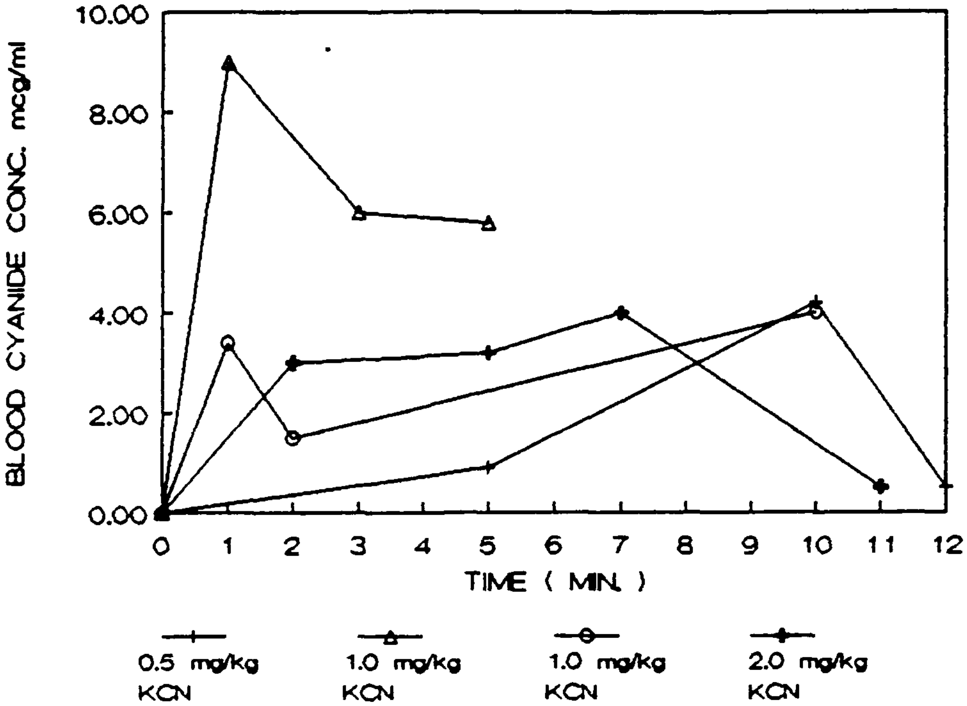


FIGURE 9

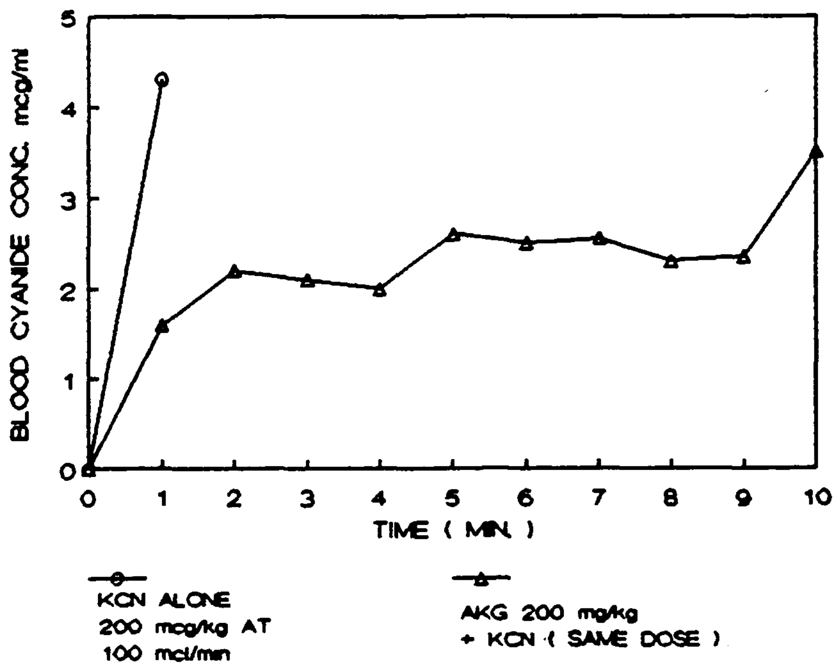
BLOOD CYANIDE LEVELS AFTER IV KCN



KCN INJECTED IV AT THE RATE OF 0.5 ml/22 SEC.

FIGURE 10

EFFECT OF AKG ON BLOOD CYANIDE LEVELS



CONSTANT KCN INFUSION WITH & WITHOUT A BOLUS DOSE OF AKG

FIGURE 11

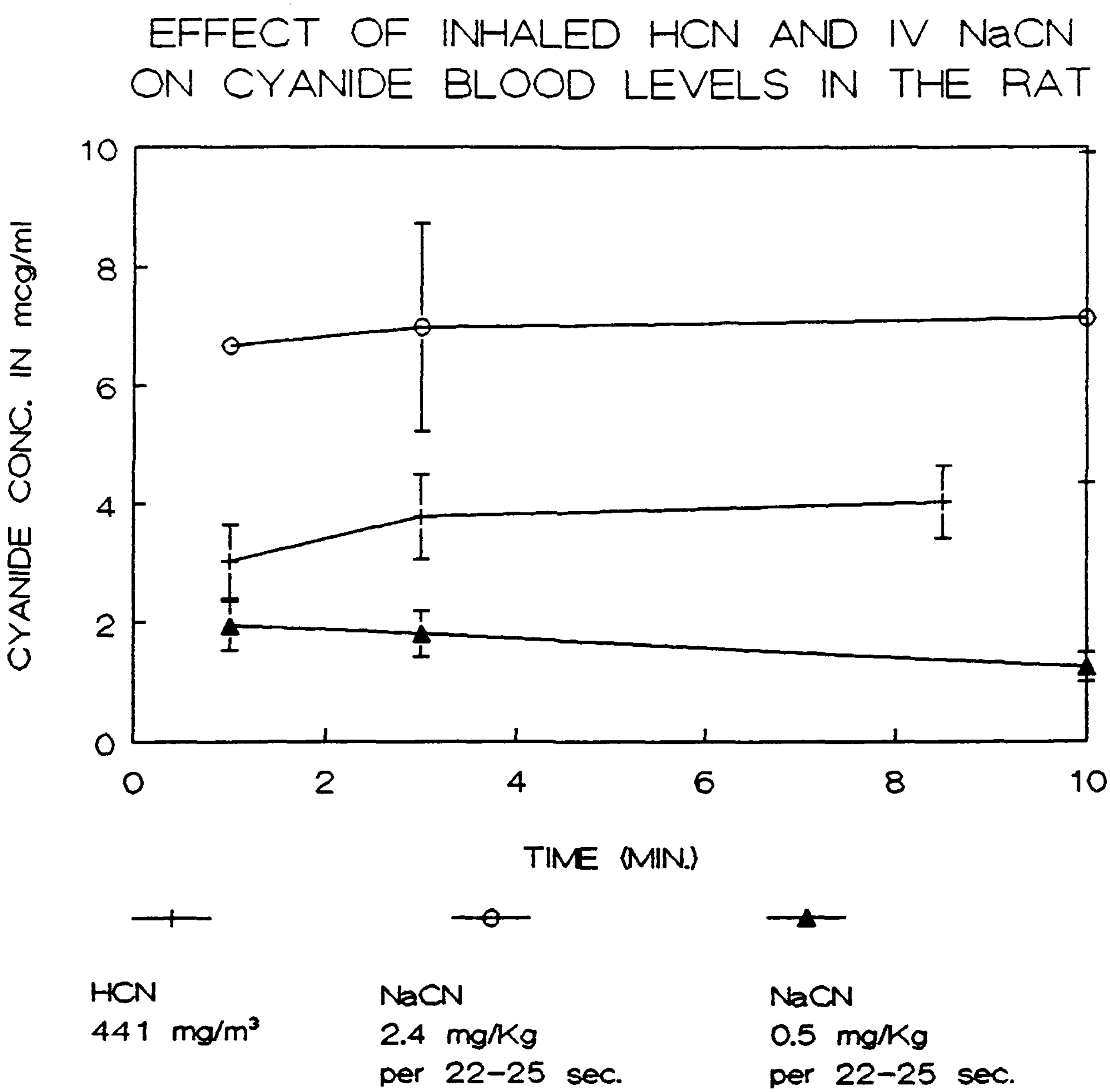
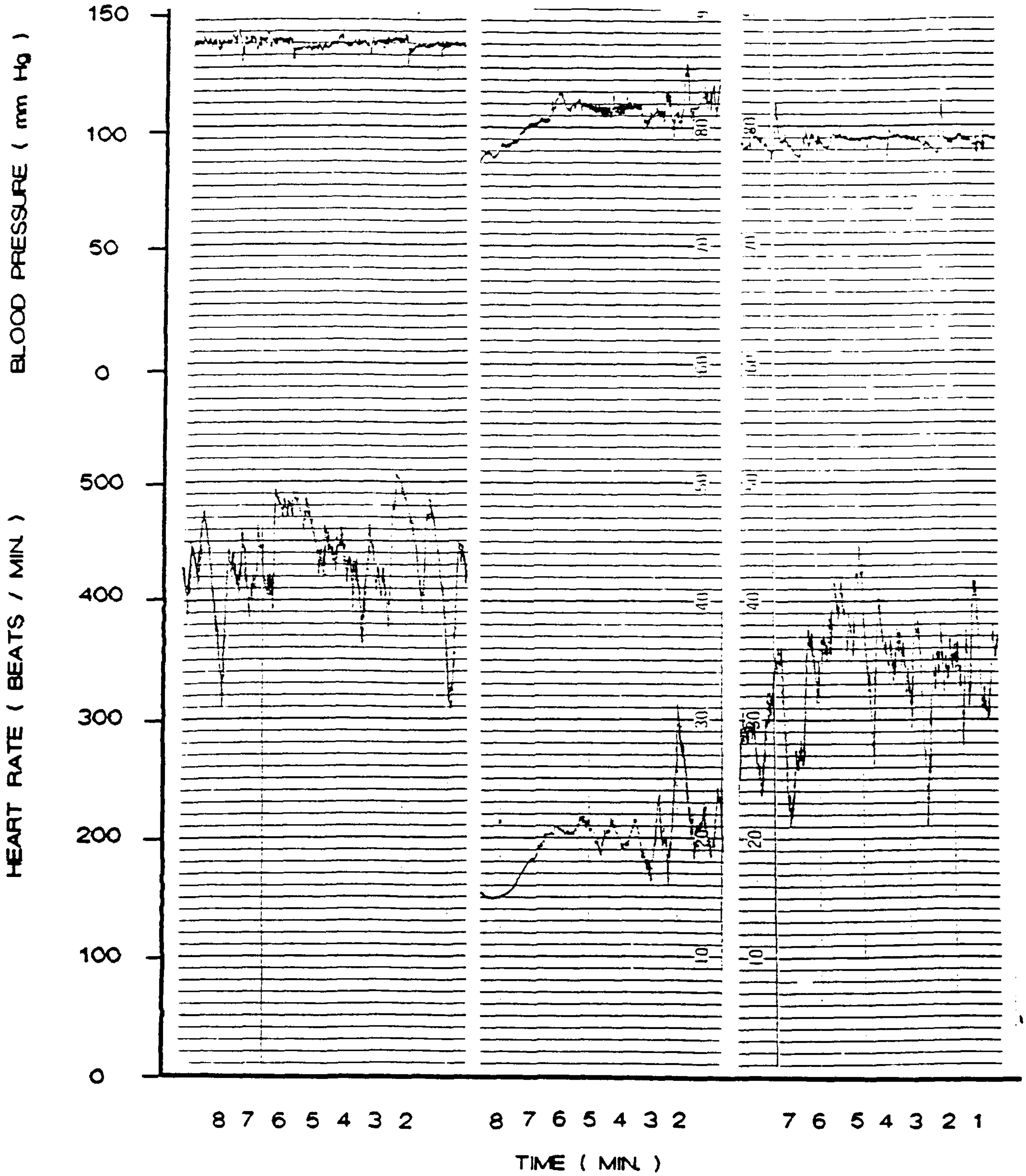

EFFECT OF INHALED HCN AND IV NaCN
ON CYANIDE BLOOD LEVELS IN THE RAT
CYANIDE CONC. IN mcg/ml
0
2
4
6
8
10
0
2
4
6
8
10
TIME (MIN.)
HCN
441 mg/m³
NaCN
2.4 mg/Kg
per 22-25 sec.
NaCN
0.5 mg/Kg
per 22-25 sec.

FIGURE 12

BLOOD PRESSURE & HEART RATE in CONSCIOUS RATS

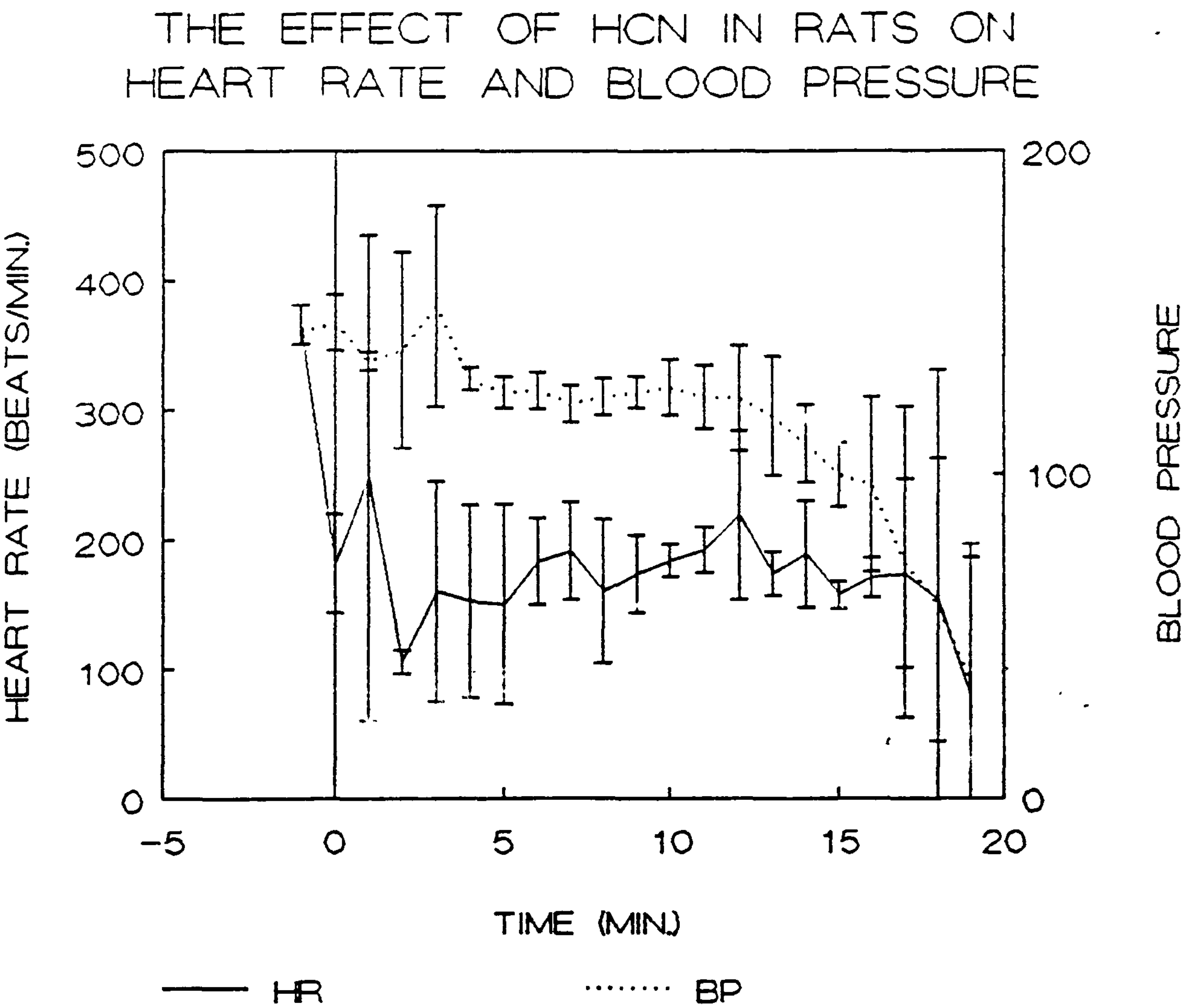


CONTROL
(AIR)

HCN
441 mg/m³

HCN 441 mg/m³
$NaNO_2$ 50 mg/kg IP

FIGURE 13


THE EFFECT OF HCN IN RATS ON
HEART RATE AND BLOOD PRESSURE
500
400
300
200
100
0
HEART RATE (BEATS/MIN.)
200
100
0
BLOOD PRESSURE
-5
0
5
10
15
20
TIME (MIN.)
HR
BP

C. Determination of Lethal Concentrations of HCN in the Inhalation Chamber.

After the desired concentration of HCN was attained and confirmed by HCN detector tubes, the HCN sensor was calibrated and a screen cage capable of containing 10 mice was quickly inserted into the chamber via the door. A concentration of 441 mg/m^3 of HCN for 3.0 minutes in the chamber typically produced an LC_{80} for mice. The chamber was continuously monitored for HCN concentration by the sensor system and HCN detector tubes. Figure 14 is a plot of the results of HCN exposure of mice. The data in Figure 14 show that an LCT_{50} of 380 mg/m^3 was determined for HCN. It is noted that the response is linear within the concentrations used. It is interesting to note (Figure 15), that the lethality of HCN parallels that of NaCN in the ranges tested. Initially, 20 randomly chosen mice were used to ascertain the lethality of the HCN concentration for the day. Therefore, before any antidotal experiments were initiated, the lethality of the HCN concentration was always established.

A compilation of 16 blank runs of 10 mice each exposed to a constant concentration of HCN indicated that the toxic inhalation chamber provided very reproducible results. The means are as follows: HCN concentration = 441 mg/m^3, CT = 1322, lethality % = 81.25 with a standard deviation of 8.06 (Figure 16).

D. Comparison of the Efficacy of Antidotes to Gaseous Hydrogen Cyanide

In general, three classes of antidotes were given to animals prior to their exposure to HCN by inhalation. The methemoglobin formers studied were sodium nitrite (SN), hydroxylamine (HA) and p-aminopropiophenone (PAPP). The non-methemoglobin forming antidote a-ketoglutaric acid (AKG) and the sulfur donor, sodium thiosulfate (ST) were also studied in this system.

The results of increasing the pretreatment doses of SN, ST and AKG are shown in Figure 17. At a chamber HCN concentration of 490 mg/m^3 and exposure for three minutes, (CT = 1470) complete protection could be afforded by 25 mg/kg of SN and 1000 mg/kg of ST while AKG at a dose of 1000 mg/kg produced a lethality reduction from 90% to 15%.

SN and ST (Table 2) were studied as standard antidotes to validate the system for HCN antidotal evaluations. In this system, SN (100 mg/kg) administered 30 minutes prior to HCN exposure reduced the HCN induced lethality from 80% to 0% and doses of 500 mg/kg of ST reduced lethality from 80% to 30%. It appears that the abilities of the methemoglobin formers SN, HA, and PAPP are dose dependent (Figure 18). PAPP is the most potent anticyanide compound of the series tested.

SN was administered in decreasing doses until protection against lethality disappeared. A dose of SN of 10 mg/kg IP. was not an effective protectant. A dose of 15 mg/kg of SN reduced the LC_{80} to LC_{30} (Figure 19) while a dose of 25 mg/kg resulted in reduction of lethality from LC_{80} to LC_0, but the animals were obviously lethargic and inactive when affected by the SN alone. Figure 19 depicts the results of experiments which were conducted to ascertain if a dose of AKG could be beneficial when added to SN. The dose of SN could be decreased until it did not affect the behavior of the animals and yet afford adequate anticyanide effects.

Eleven (11) prospective anticyanide chemicals provided by USAMRICD were evaluated in this system. The results of these studies is shown in Table 2. The chemicals were injected IP in the dosages and in combinations recommended by USAMRICD. Additional dosages of these chemicals were included in the study in order to compare potencies of anticyanide compounds. All of these chemicals as tested exhibited some protection against the HCN concentration used. However, only ICD1104, ICD1115, and AKG showed sufficient protection to act as a prophylactic against HCN. The compound ICD1115, apparently provided only a temporary protection as more mice were dead at 72 hours than at 0 or 24 hours.

The non-methemoglobin former AKG and sulfur donor were found to be less effective protectants against inhaled cyanide than parenterally administered cyanide. For example, 500 mg/kg of AKG repeatedly reduced the LD_{80} of IP injected NaCN to LD_0. However, a dose of AKG of over 1000 mg/kg was required to afford the same protection against inhaled HCN. The sulfur donor, ST, also required increased dosage to protect the animals against inhaled HCN. As seen in Figure 20 a dose of 1000 mg/kg of ST was required to reduce the LC_{80} to LC_0.

FIGURE 14

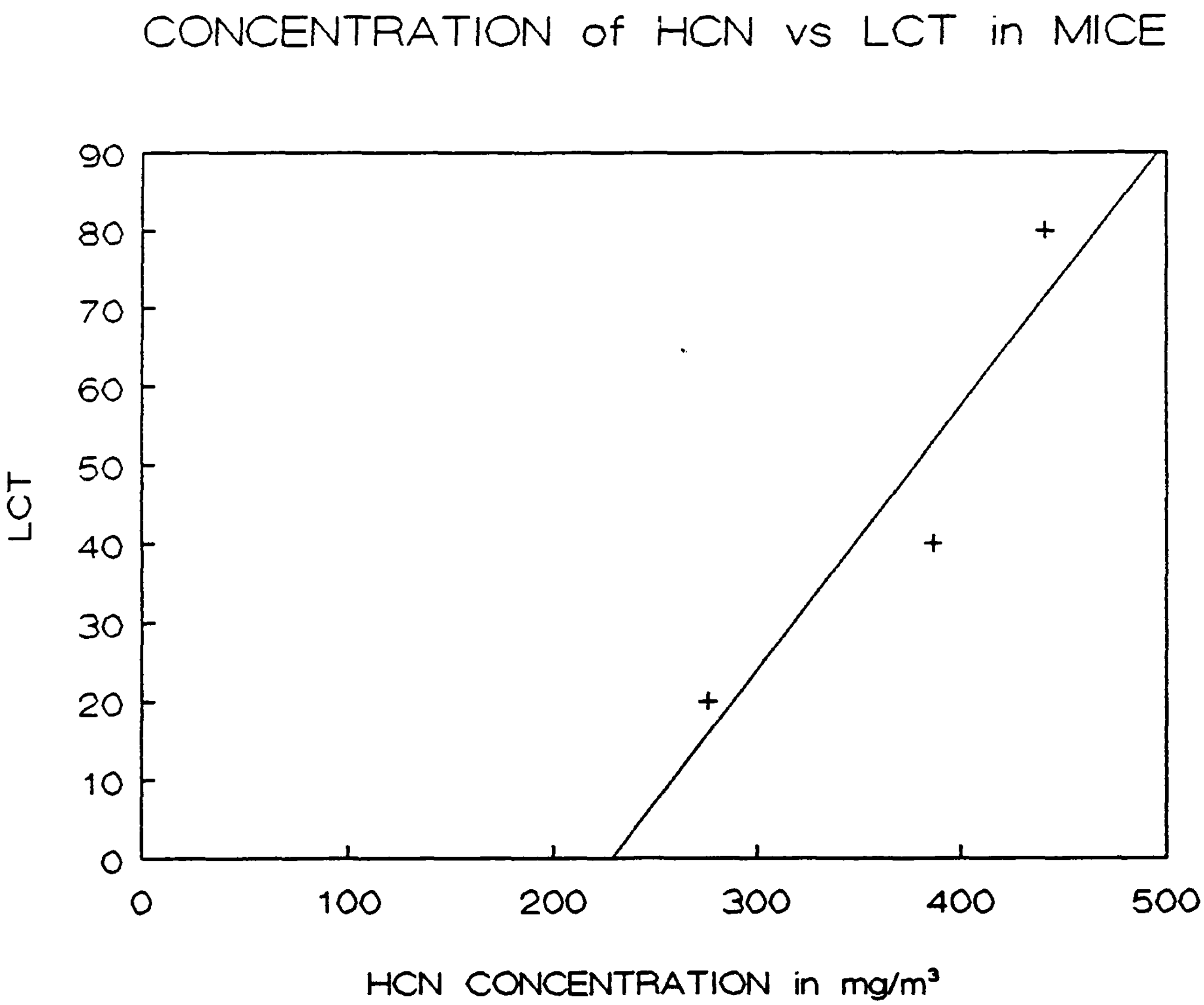
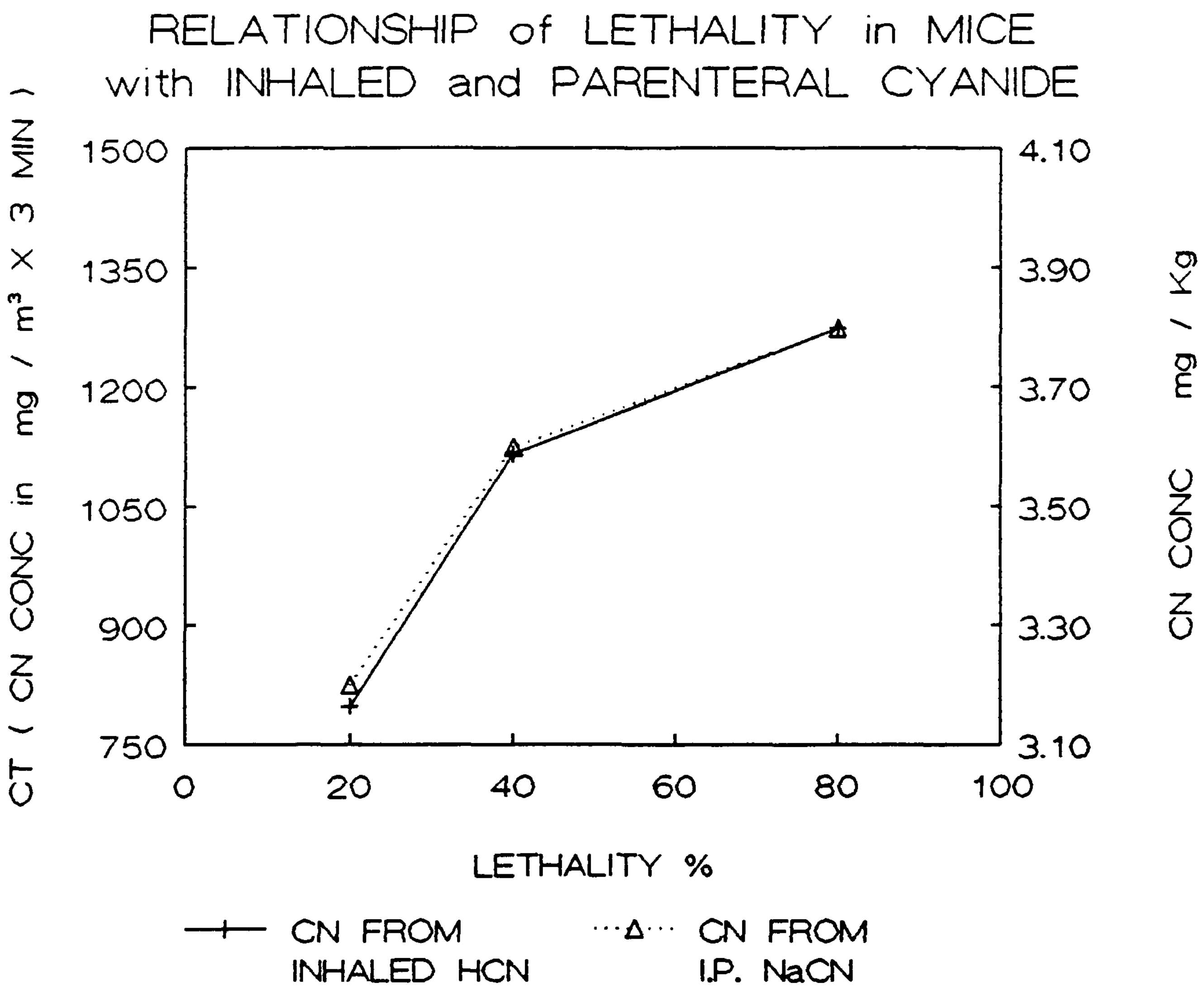
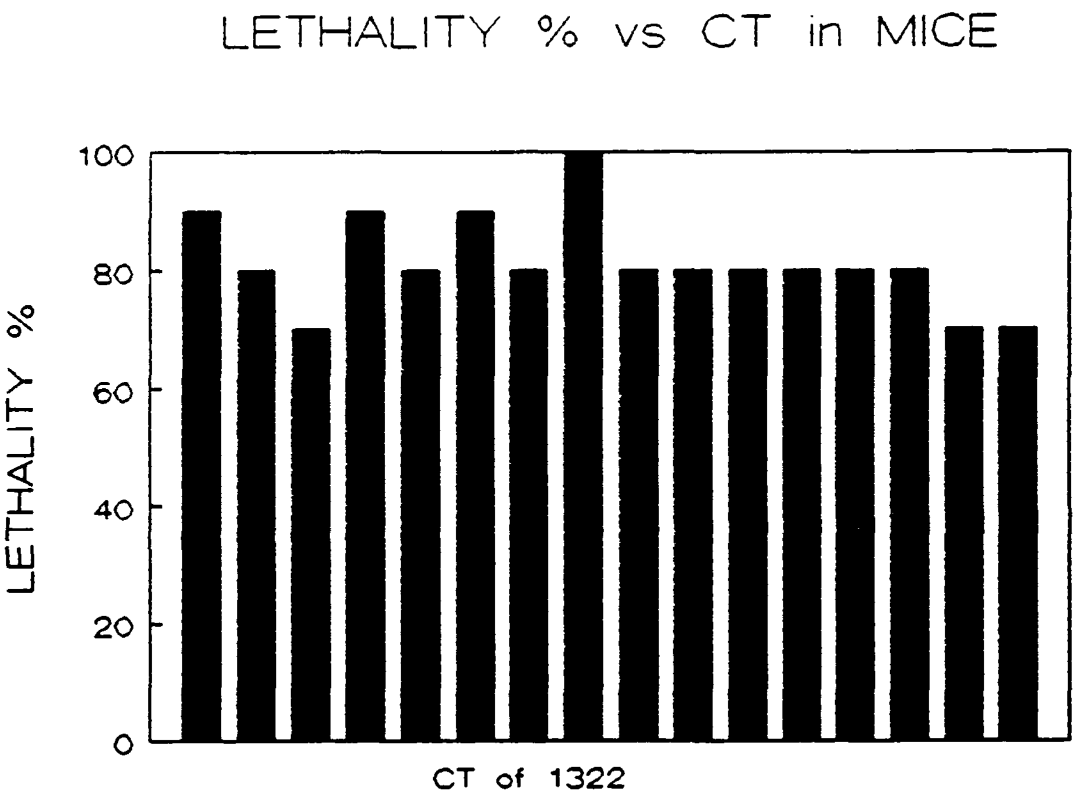

CONCENTRATION of HCN vs LCT in MICE
LCT
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
HCN CONCENTRATION in mg/m³

FIGURE 15



RELATIONSHIP of LETHALITY in MICE
with INHALED and PARENTERAL CYANIDE
CT (CN CONC in mg / m³ X 3 MIN)
1500
1350
1200
1050
900
750
4.10
3.90
3.70
3.50
3.30
3.10
CN CONC mg / Kg
0
20
40
60
80
100
LETHALITY %
CN FROM INHALED HCN
CN FROM I.P. NaCN

FIGURE 16



A CT of 1322 (HCN concentration in mg / m^3 times TIME in minutes) typically resulted in an 80% Lethality

FIGURE 17

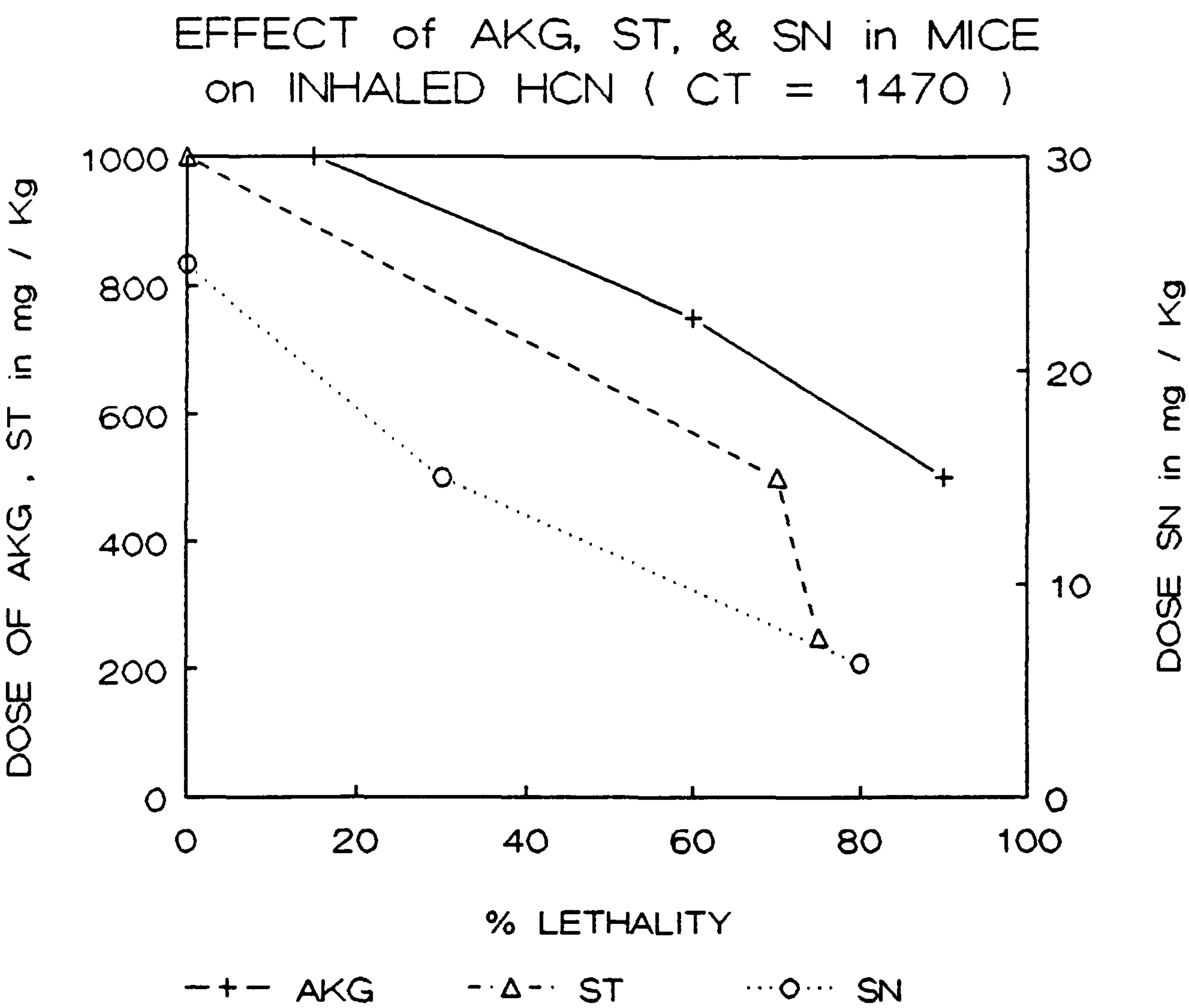

EFFECT of AKG, ST, & SN in MICE
on INHALED HCN (CT = 1470)
DOSE OF AKG , ST in mg / Kg
DOSE SN in mg / Kg
1000
800
600
400
200
0
30
20
10
0
0
20
40
60
80
100
% LETHALITY
—+— AKG
- -Δ- - ST
···O··· SN

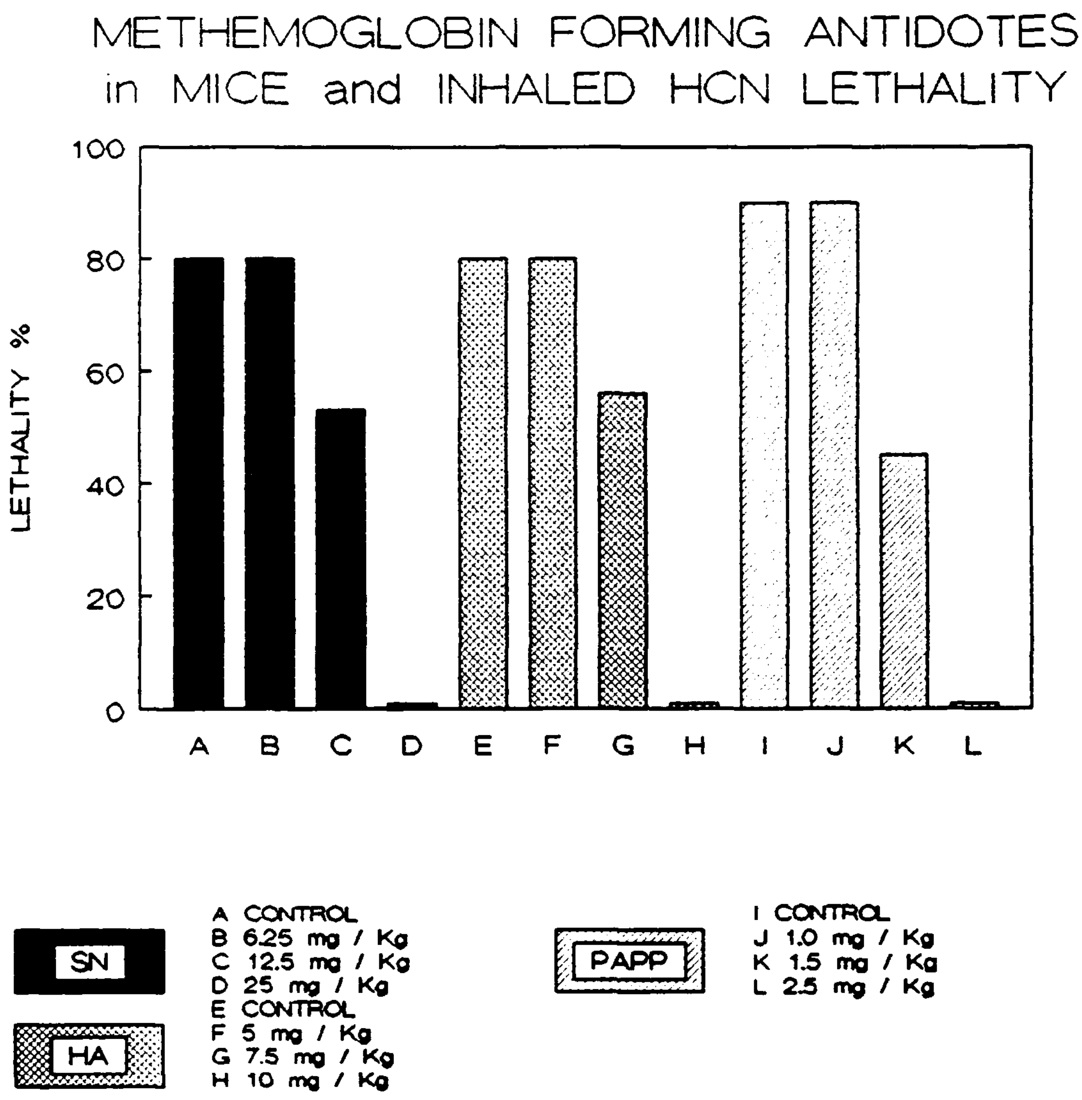
Table 2

Results of Tests of Antidotes

Antidote	Dose mg/kg	No. mice	HCN conc. mg/m^3(S.E.M)	LETHALITY % (S.E.M) 0 hrs	24 hrs	72 hrs	Decrease in lethality %
SN	50.0	10	496	0	0	0	80
SN	25.0	10	496	0	0	0	80
SN	15.0	10	496	30	30	30	15
SN	12.5	30	496	53	53	53 (3)	27
SN	10.0	10	441	80	80	80	0
SN	6.25	20	496	80	80	80 (0)	0
ST	1000	10	452	0	0	0	75
ST	500	20	496	70	70	70 (0)	5
ST	250	8	496	75	75	75	0
AETS	5	20	441	100	100	100 (0)	0
HA	10	10	441	0	0	0	80
HA	7.5	9	441	56	56	56	24
HA	5.0	20	436	50	50	50 (10)	30
ICD1021	107.5*	20	491	40	40	40 (20)	45
ICD1297	30*	20	414	0	65	80 (10)	10
ICD1297	15*	20	441	5	35	75 (15)	15
ICD1350	34.1	20	430	25	30	30 (10)	50
ICD1104	48.9	20	436	5	5	5 (5)	75
ICD1104	24.5	10	496	0	0	0	80
ICD1104	12.2	20	496	20	20	20 (0)	60
ICD1104	6.1	20	441	65	65	65 (5)	20
ICD1115	72.0*	10	441	50	50	60	35
ICD1115	48.5*	28	445	22	26	38 (28)	42
ICD1115	24.3	10	413	60	60	80	20
ICD1171	75*	39	441	30	58	58 (42)	17
ICD1171	37.5*	20	469	25	50	50 (0)	25
AKG	1000	20	455	15	15	15 (5)	75
AKG	750	10	441	60	60	60	20
AKG	500	10	496	90	90	90	0
AKG	250	20	457	90	90	90 (0)	0
AKG	250	40	464 (59)	73	73	73 (7)	7
AETS	5						
AKG	500	60	437 (18)	72	72	72 (18)	18
AETS	50						
AKG	500	20	444 (2)	25	25	25 (5)	55
SN	10						
AKG	250	30	494 (20)	67	67	67 (3)	13
AETS	5						
HA	10						
AKG	250	10	441	70	70	70	10
AETS	5						
HA	5						
SN	12.5	7	496	43	43	43	27
ST	250						
SN	25	10	496	0	0	0	80
ST	500						
AKG	250	10	468	20	20	20	70
ST	500						

*-produced lethargy at dose indicated - Compounds ICD 1021 and ICD 1297 were dissolved in multisol and PEG, respectively. Both solvents produced lethargy at a dose of .1ml/10g mice weight.

FIGURE 18



A CONTROL
B 6.25 mg / Kg
C 12.5 mg / Kg
D 25 mg / Kg
E CONTROL
F 5 mg / Kg
G 7.5 mg / Kg
H 10 mg / Kg

I CONTROL
J 1.0 mg / Kg
K 1.5 mg / Kg
L 2.5 mg / Kg

FIGURE 19

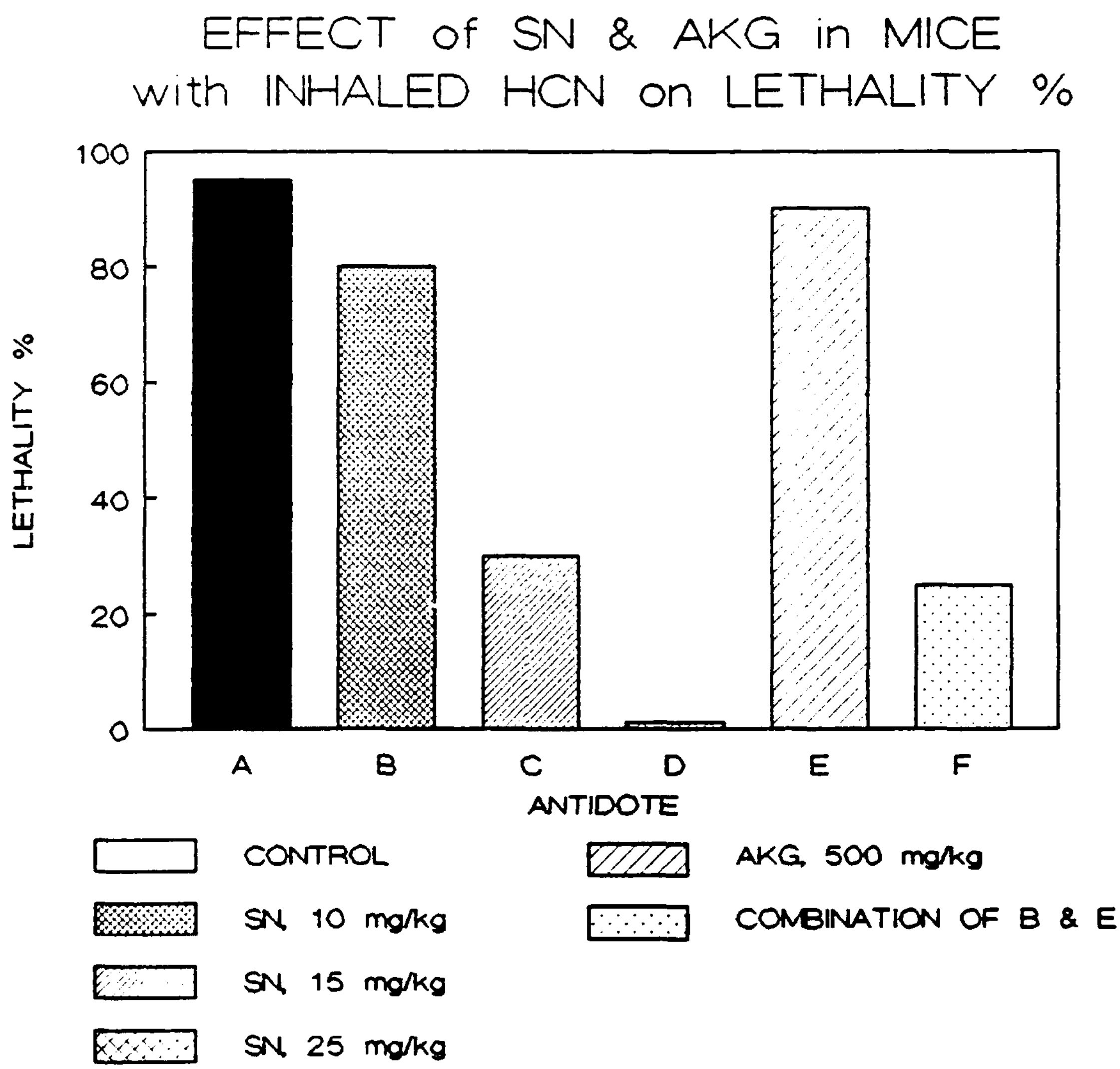
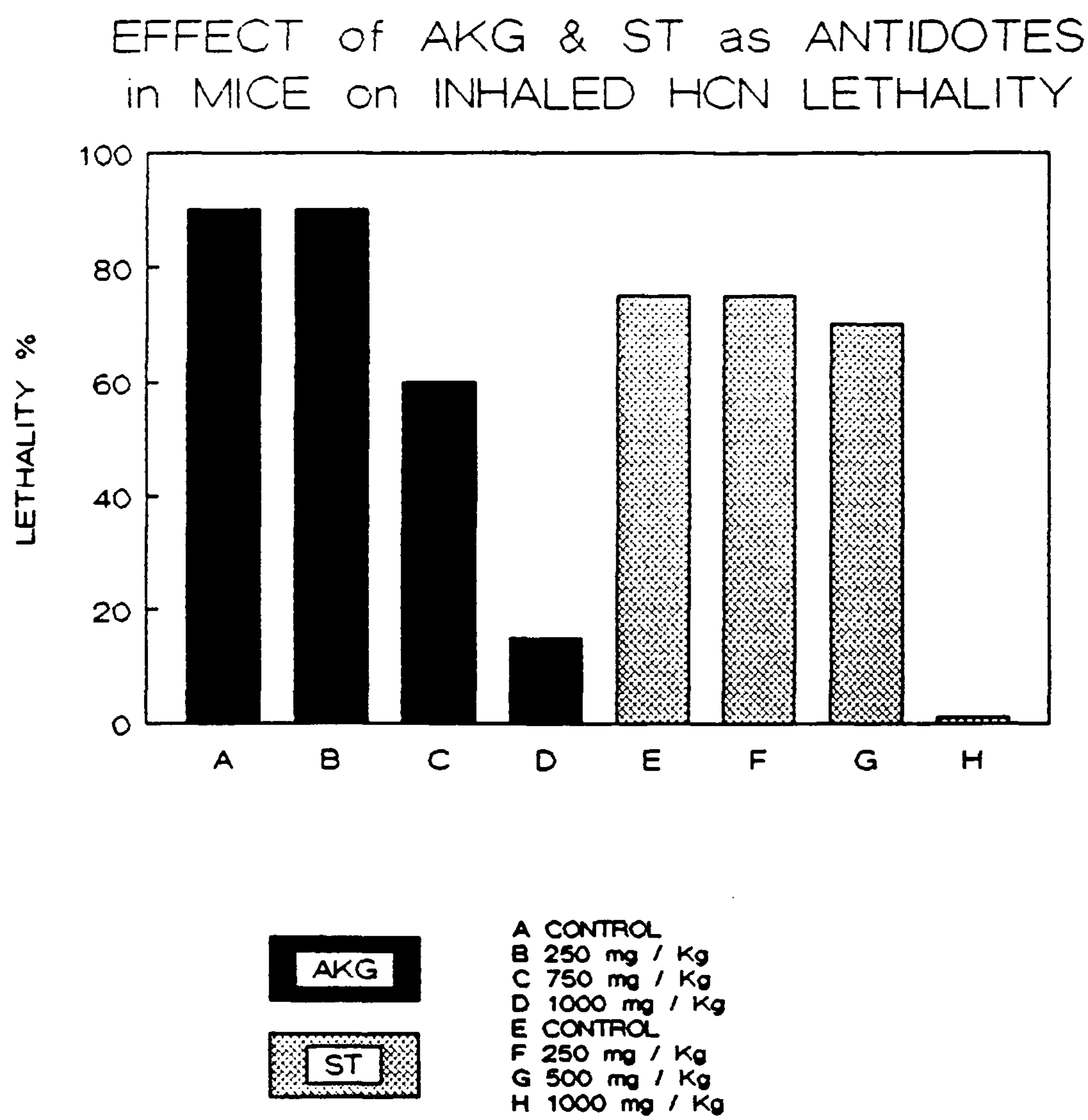

EFFECT of SN & AKG in MICE
with INHALED HCN on LETHALITY %
LETHALITY %
100
80
60
40
20
0
A
B
C
D
E
F
ANTIDOTE
CONTROL
SN, 10 mg/kg
SN, 15 mg/kg
SN, 25 mg/kg
AKG, 500 mg/kg
COMBINATION OF B & E

FIGURE 20


EFFECT of AKG & ST as ANTIDOTES
in MICE on INHALED HCN LETHALITY
LETHALITY %
100
80
60
40
20
0
A
B
C
D
E
F
G
H
AKG
ST
A CONTROL
B 250 mg / Kg
C 750 mg / Kg
D 1000 mg / Kg
E CONTROL
F 250 mg / Kg
G 500 mg / Kg
H 1000 mg / Kg

The methemoglobin formers SN and HA provided protection against HCN at relatively low doses. At recommended dosages, ICD 1297 and ICD 1350 (methemoglobin formers) provided little or no protection as seen in Figure 21 and all methemoglobin formers produced some degree of lethargy at effective doses.

Some combinations of antidotes to the effects of inhaled HCN greatly reduced the lethality. As seen in Figure 22, a dose of 10 mg/kg of SN or 500 mg/kg of AKG offered little or no protection against inhaled HCN. However, the same doses combined reduced the lethality from 80 to 20%. Combinations of ST and SN as well as AKG and ST were also studied and proved to be effective antidotes.

V. CONCLUSIONS

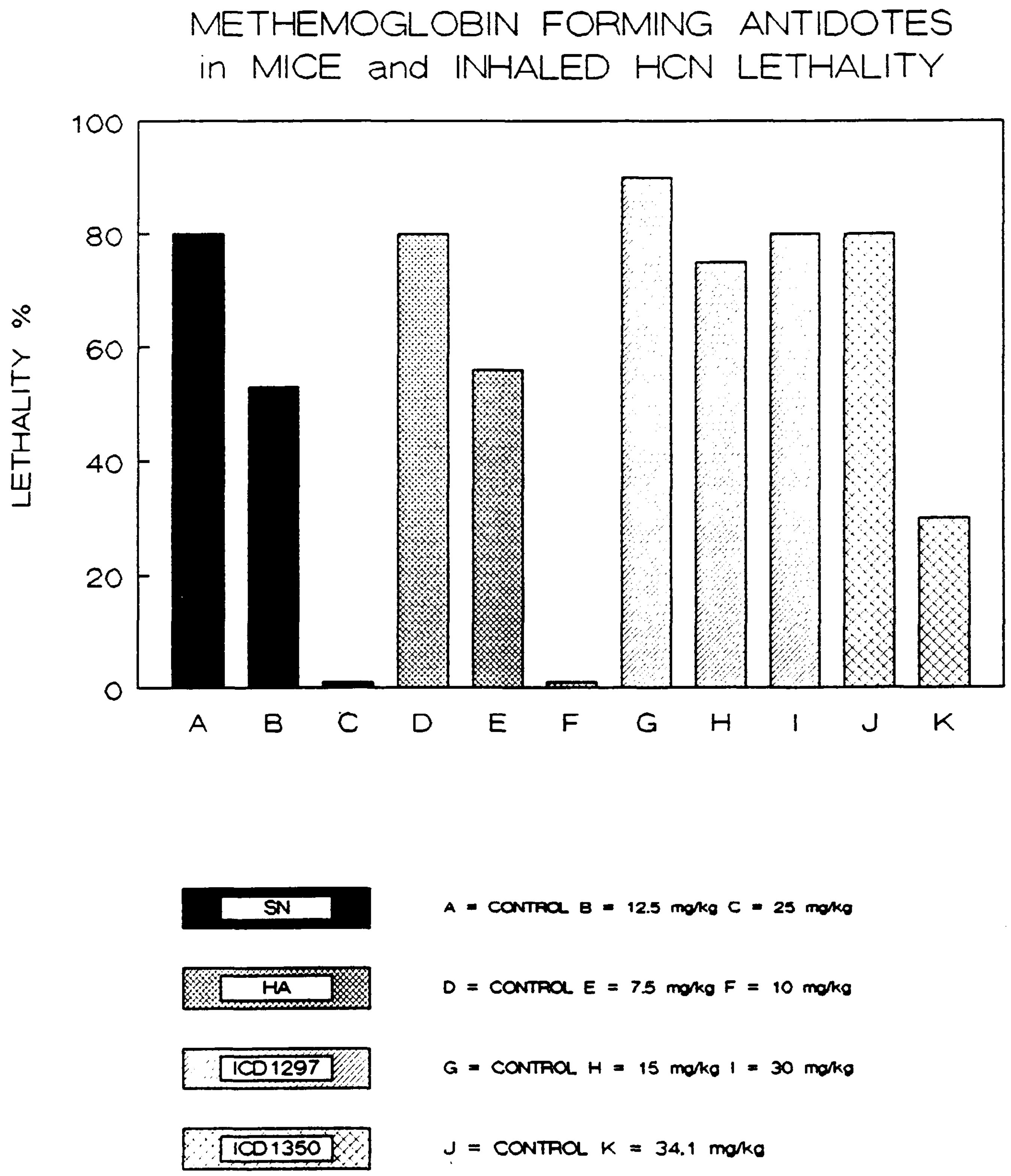
The mechanisms are in place to accumulate much needed information in the area of antidoting the effects of inhaled cyanide. Not only can we learn much about the mechanism of cyanide poisoning, i.e., cardiovascular effects, lethal blood cyanide levels, and effects of hypoxia, but also antidotes can be evaluated against gaseous HCN. This is extremely important since this form of cyanide is probably the most likely to be delivered as a chemical warfare agent. It appears that military relevance would require the accumulation of information in developing a militarily suitable and effective antidote for cyanide. As previously mentioned, antidotes to HCN poisoning vary in their effectiveness when compared to ionic cyanide exposures. Much previous work on cyanide antidotes done by this investigator and others on ionic cyanide was thought to be applicable to HCN. Our findings now indicate this assumption is not always valid.

Since HCN remains in the arsenals of many developing countries, the danger to our service personnel continues to be a problem. While the methemoglobin formers have shown promise in being an effective antidotes to HCN, the actual percentage of formation of methemoglobin varies widely on an individual basis. Death from the uncertain dose which produces excess methemoglobin formation continues to be the major problem encountered with this regimen. Additional chemicals which produce methemoglobin need to be screened to determine if there is one which will produce the desired formation at a predictable level. The a-keto acids, while showing some antidotal activity with HCN, are required in large doses. Derivitization of these acids could possibly produce a nontoxic compound which may be effective against HCN poisoning. The fact that thiosulfates also produce antidotal activity should not be overlooked. Perhaps a thiosulfate derivative of an a-keto acid may provide protection to HCN. Also, the affinity of cyanide for the $Fe+3$ ion should be investigated. A good antidote should be effective sufficiently to "blunt" the lethal effects and allow survival so that medical management can be initiated, or autoinjection of postexposure antidotes can be accomplished. Preferably, an antidote should be innocuous, prophylactic in its action and orally administrable. At this point, AKG is the only effective antidote evaluated in our laboratory which exhibits all of these properties.

A model with a purpose to study the toxic effects of HCN by inhalation and to evaluate the efficiencies and relative potencies has been established. Several chambers and accompanying mechanics of administration of HCN were designed and evaluated. This model was specially designed to be used to study the effects of HCN at different concentrations and to evaluate antidote effectiveness of antidotes with the pre- and post-exposure of mice and rats to HCN. In addition, pulmonary and cardiovascular changes in the rat can be monitored. This allows the determination of efficacy of antidotes more discretely and monitors toxic effects other than lethality.

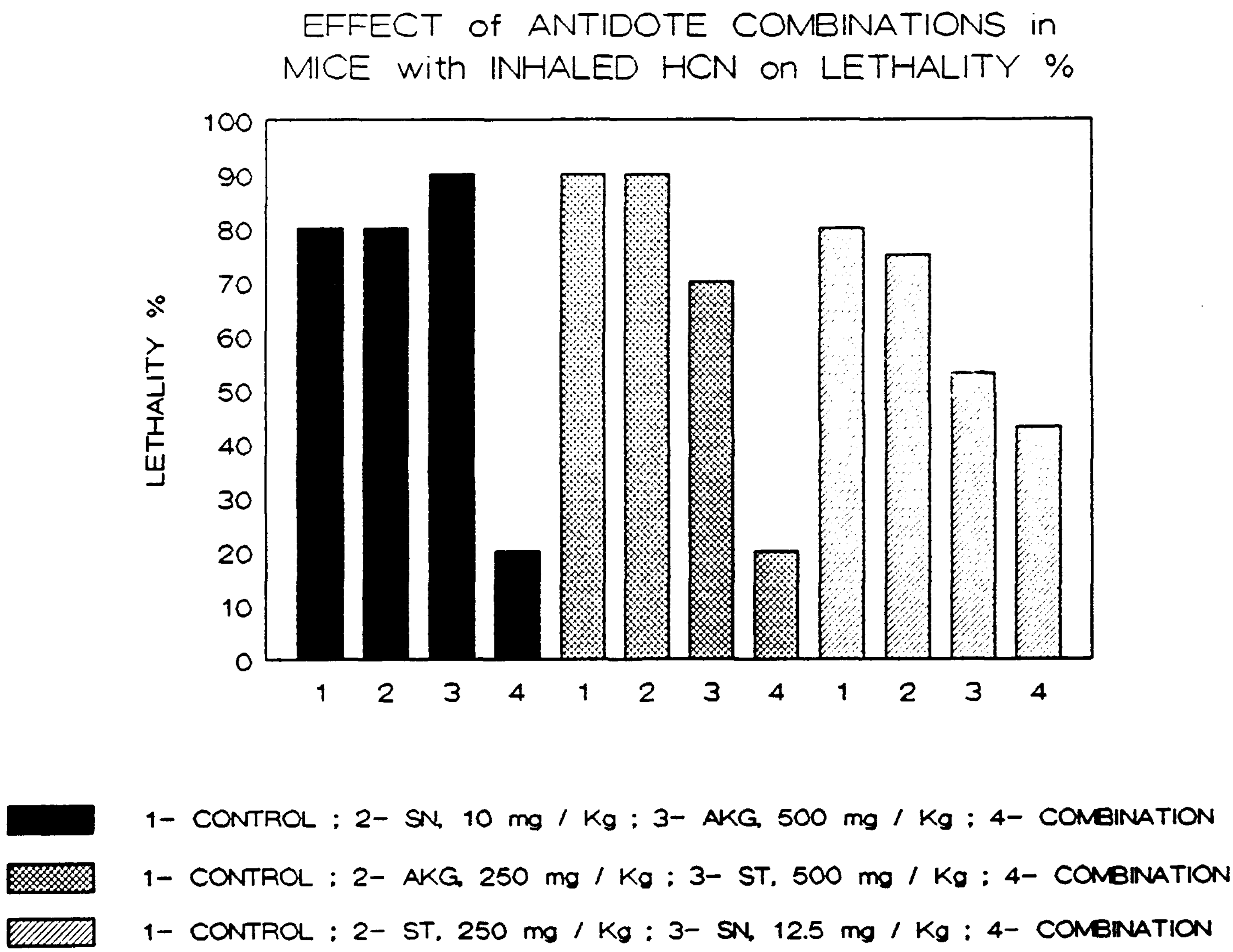
Since HCN is so acutely toxic, minor changes in the HCN concentration in the chamber during exposure can result in major changes in the toxic effects that are produced. In order to establish and maintain the HCN concentration in the chamber, two methods of supplying HCN were attempted: (1) the reaction of a cyanide salt and sulfuric acid in molar quantities and (2) purchase of commercially available HCN mixture. Problems developed in this work with commercially available HCN. Two different tanks of HCN/He were received that were much in error in concentration of HCN, even though they had supposedly been analyzed by the vendor (Matheson). Every effort was made in this model to establish and maintain a stable HCN concentration.

FIGURE 21



METHEMOGLOBIN FORMING ANTIDOTES
in MICE and INHALED HCN LETHALITY
LETHALITY %
0
20
40
60
80
100
A
B
C
D
E
F
G
H
I
J
K
SN
A = CONTROL B = 12.5 mg/kg C = 25 mg/kg
HA
D = CONTROL E = 7.5 mg/kg F = 10 mg/kg
ICD 1297
G = CONTROL H = 15 mg/kg I = 30 mg/kg
ICD 1350
J = CONTROL K = 34.1 mg/kg

FIGURE 22


EFFECT of ANTIDOTE COMBINATIONS in MICE with INHALED HCN on LETHALITY %
LETHALITY %
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 1 2 3 4 1 2 3 4
1- CONTROL ; 2- SN, 10 mg / Kg ; 3- AKG, 500 mg / Kg ; 4- COMBINATION
1- CONTROL ; 2- AKG, 250 mg / Kg ; 3- ST, 500 mg / Kg ; 4- COMBINATION
1- CONTROL ; 2- ST, 250 mg / Kg ; 3- SN, 12.5 mg / Kg ; 4- COMBINATION

In order to establish and maintain an accurate and precise HCN concentration, the following mechanisms were used: (1) assayed HCN/helium mixture, (2) measured gas flows to deliver a definite concentration of HCN from the tank, (3) rotameters and mixer to deliver regulated gas flows of HCN and breathing air to the chamber, (4) cyanide electrode in the chamber for constant HCN concentration monitoring, (5) HCN detector tubes to determine HCN concentration in the effluent flow from the chamber, (6) periodic chemical or gas chromatographic analyses of the HCN content in the chamber.

Numbers (4) and (5) are necessarily rapid measurements in which the results are instantaneous or quick (<2 min). The results of these procedures is a system in which the HCN concentration is highly reproducible and constant for comparison of antidotes if potency ratios are to be meaningful.

Validation of the chamber was supported by the use of SN and ST as standard antidotes for HCN. Exposure to a concentration of 441 mg/m^3 of HCN for 3 minutes was selected since it produced 80% lethality. Different doses of antidotes were evaluated against this concentration. The methemoglobin-forming antidote SN, when administered at a dose of 100 mg/kg IP, 30 minutes prior to exposure resulted in an inactive, lethargic response in mice and was sometimes lethal. However, 100% protection to 441 mg/m^3 HCN was afforded at this dose.

When the dose of SN was in mice reduced to 10 mg/kg IP, no protection was provided. ST was not significantly protective against an LCT_{80} of HCN until the dose of ST was increased to 1000 mg/kg. The combination of SN (12.5 mg/kg) and ST (250 mg/kg) afforded a reduction in lethality from 70% to 43%.

It is interesting that the non-methemoglobin-forming antidote AKG which provided complete protection against an LD_{80} of NaCN at a dose of 250 mg/kg required a fourfold increase in dosage in order to provide significant protection against an LCT_{80} level of HCN. A combination of SN and AKG might prove useful as this would allow reduction of the undesired, disabling SN induced side effects while providing protection.

Thus, from these data it would appear the methemoglobin-forming antidotes tested are more effective than non-methemoglobin-forming antidotes against inhaled HCN. The explanation for this result is unclear; it could be that an alteration of cerebral blood flow reduces the delivery of HCN to the respiratory center. Additional research is needed in this area. It is difficult to assign these effects to the formation of methemoglobin alone.

These experiments indicate that inhaled HCN is more toxic than the intravenously administered cyanide salt. The onset of toxic symptoms is quicker with a lower blood CN level attained, than intravenously administered cyanide. Lethality occurs at a lower CN blood level resulting from inhaled HCN than with the parenterally administered cyanide salt.

Cardiovascular effects of HCN (tachycardia then bradycardia with arrhythymias) were observed. These effects are characteristic of toxicity and appear quicker and are equally as severe as intravenously administered cyanide salts.

Other methemoglobin-forming anticyanide agents such as PAPP and DMAP should be evaluated against HCN and relative potencies determined for comparison with other methemoglobin-forming antidotes. Other non-methemoglobin forming antidotes such as HCB and cobalt edetate (Kelocyanor®) should be evaluated for anti-HCN activity. These evaluations should be carried out against at least three different concentrations of HCN. Then, anticipated battlefield concentrations could be challenged.

These studies show that there are toxicological differences in the mechanism of inhaled HCN and parenteral doses of CN which should be examined. These significant differences affect the requirements for effective antidotal activity, particularly of a chemoprophylactic agent. It would also appear that a chemoprophylatic agent is essential if survival of acute HCN exposure is to result. The antidote must be in place in the target organs in order to antagonize the lethal effects of high concentrations of inhaled HCN. Compounds (identified by number only) were evaluated against an LCT_{80} HCN in the chamber, at the doses recommended. None of these compounds except ICD 1140, exhibited significant anticyanide activity without severe toxic effects

due to the test antidote itself. The system proved that it can provide very useful data in the search for a chemoprophylactic antidote for inhaled cyanide induced toxic effects.

VI. LITERATURE CITED

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VII. ABBREVIATIONS USED IN REPORT

AETS = Aminoethane Thiosulfonate
AKG = A-ketoglutaric acid
BKG = B-ketoglutaric acid
BP = blood pressure
CN or CN^- = cyanide ion
DMAP = dimethylaminophenol
HA = hydroxylamine
HCB = hydroxocobalamin
HCN = hydrogen cyanide
HR = heart rate
IM = intramuscular (ly)
IP = intraperitoneal (ly)
IV = intravenous (ly)
KCN = potassium cyanide
mA = millamperes
MBP = mean blood pressure
MHB = methemoglobin
NaCN = sodium cyanide
NON-MHB = non-methemoglobin
PAPP = p-aminopropiophenone
SN = sodium nitrite
ST = sodium thiosulfate

VIII. APPENDIX

A. Publications Resulting from the Research of this Project

Hume, A.S., S. Whitney, M. Dulaney, Jr., M.A. Brumley, and J.T. Willis. Effective antagonism of cyanide (CN) induced lethality by oral alpha-ketoglutaric acid (AKG). J. Miss. Acad. Sci. 33: 60, 1988.

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Hume, A.S., Baker, M. and A. Markow. The effects of alpha-ketoglutaric acid (AKG) on cyanide (CN) induced acidosis. Proceedings of Fifth Annual Chemical Defense Bioscience Review, Aug. 15-17, 1989, Columbia, MD.

Hume, A., M. Dulaney, T. Collins, and J. Crout. Alpha ketoglutaric acid (A-KG) and its antagonism of toxic of postassium cyanide (KCN) V. Intr. Congr. of Tox. Brighton, England, 487, 1989.

Hume, A., J. Mozingo and M. Dulaney. The structure activity relationship of keto acid to act as antidotes against the nucleophilic poisons, cyanide (CN^-) and sulfide ($S^=$) V Inter. Cong. Tox. Brighton, England, 1989.

Dulaney, M., and A. Hume. The efficacy of alpha-ketoglutarate (AKG), 2-aminoethane thiosulfonate (2-AETS) pretreatment and hydroxylamine (HA) post-treatment as cyanide antidote in the mouse. Toxicologist, 11, 31, p. 108, 1991.

Hume, A., M. Dulaney, Jr., and J. Mozingo. The efficacy of alpha-ketoglutaric acid and methemoglobin formers against gaseous cyanide. Toxicologist, 11, #1, p. 107, 1991.

Mozingo, J., M. Dulaney, Jr., and A. Hume. A dynamic inhalation chamber for studying antidotes to asphyxiating gases. Toxicologist, 11, #1, p. 107, 1991.

Dulaney, M., M. Brumley, J. Willis, A. Hume. Protection against cyanide toxicity by oral alpha-ketoglutaric acid (Accepted for publication Vet and Hum. Tox., March, 1991).

B. Personnel Supported Under this Project.

1. Dr. James Mozingo
2. Ms. Johnette Gothard
3. Ms. Angie Hembree

SUPPLEMENTARY

INFORMATION

ERRATA AD A 244 070

Results of a study in which antidotal agents were administered on a molar basis are listed in Table 1. Kelocyanor, hydroxocobalamin, alpha-ketoglutaric acid, sodium nitrite and sodium thiosulfate were administered in 2:1, 4:1, 5:1 and 20:1 molar ratios of antidote: cyanide. All antidotes were effective in protection against lethality at LD_{40}, LD_{70} and LD_{90} doses of NaCN. However, at a 20:1 ratio, the required 200 mg/kg dose of sodium nitrite was lethal in 100% of the animals tested.

On an equimolar basis, AKG, dicobalt edetate (CoEDTA), and HCB provided complete protection at these dosages of NaCN administered parenterally.

In Figure 7, AKG is shown to increase the LD_{50} of potassium cyanide (KCN) from 6.47 to 10.97 mg/kg at a dose of 200 mg/kg IP. AKG was compared to HCB at a cyanide dose of 9.70 mg/kg and the protection was approximately the same. Also, when sodium thiosulfate is added to AKG and HCB, the LD_{50} of cyanide is increased to 26.05 and 25.85 mg/kg, respectively (Figure 8). It is apparent from these results that AKG and HCB are quite comparable in efficacy as antidotes for the toxic effects of cyanide.

Blood cyanide levels in rats were obtained after intravenous administration of 0.5, 1.0 and 2.0 mg/kg of KCN (Figure 9). In Figure 10, the single value for KCN alone represents a blood sample collected at the time of death of the animal. The wide variability in the results as observed in figures 9 and 10 are expected since the doses and rate of injection are different. However, some significance can be assigned to the survivability and constant blood CN' level in the AKG treated animals.

Additional studies on rats with NaCN and HCN were accomplished. In the experiments, rats were injected IV over 22-25 seconds with two concentrations of NaCN. In the study, 2.4 mg/kg of NaCN was injected to one group and 0.5 mg/kg to the other to produce the following mean cyanide blood level concentrations.

Conc. of NaCN	CN blood level 1.0 min	CN blood level 3.0 min	CN blood level 10.0 min
2.4 µg/kg	6.67 µg/ml	6.98 µg/ml	7.13 µg/ml
0.5 µg/kg	1.94 µg/ml	1.81 µg/ml	1.25 µg/ml

With NaCN, all rats recovered. However, this was not the case with inhaled HCN. Even though cyanide blood levels were much lower than one group of the NaCN injected rats, all rats receiving the inhaled HCN died within 8.5 minutes (Figure 11).

Conc. of HCN	CN blood level 1.0 min	CN blood level 3.0 min	CN blood level 8.5 min
441 mg/m^3	3.03 µg/ml	3.78 µg/ml	4.03 µg/ml

Cardiovascular monitoring procedures have been developed in our laboratory. Rats were catheterized as described elsewhere. The arterial catheter was connected via transducer to a 2 pen recorder. A typical control was observed in Figure 12, in which HR and MBP were monitored. The catherized rats in this experiment were allowed to equilibrate in the holding tube and then were exposed to air or HCN. A concentration of 441 mg/m^3 HCN produced a rapid drop in heart rate with the blood pressure first increasing and then rapidly decreasing until death at approximately 8 minutes. The antidote, SN, was given 30 minutes prior to HCN exposure and produced an overall lowering of blood pressure and heart rate but offered the animal complete protection for over 20 minutes. The rapid decrease in heart rate and the slight increase and then rapid decrease in blood pressure appeared to be typical of rats exposed to HCN (Figure 13).

AD-A 244 070